

**Nuclear matrix attachment regions associated with chromosomal translocations in cancer.** *M. Goldman, M. Wuerth.* Dept Biol, San Francisco State Univ, San Francisco, CA.

Most cancers display genomic instability, including chromosome rearrangements. An understanding of the mechanism of cancer-associated gene rearrangement requires an accurate map of key features of chromatin that might be important in determining the location of breakpoints. We have analyzed chromosomal regions associated with recurrent translocations found in human cancers for the occurrence of nuclear matrix attachment regions (MARs or SARs). DNA sequences for genes associated with recurrent chromosome translocations found in human cancers were obtained from the National Cancer Institutes Cancer Genome Anatomy Project.

One hundred twelve genes associated with recurrent chromosome translocations found in human cancers were analyzed using MARFinder. Twenty-eight of those genes (25%) were predicted by MARFinder to have MARs in the immediate vicinity. Genes associated with translocations found in human cancers with predicted MARs were found on every chromosome except for chromosome 7. Nine of the 28 genes associated with chromosome translocations found in human cancers with predicted MARs were transcription factors or activators, and seven coded for zinc finger proteins. As a control, forty human housekeeping genes were also analyzed. Seven of those genes (17%) were predicted to have MARs, suggesting the MARs may be more highly represented in the vicinity of translocations. Showing an association between MARs and genes associated with chromosome translocation in human tumors could provide an important clue to understanding cell proliferation in human cancers. An association between MARs and translocation sites may provide insight into the mechanisms of chromosome translocation. The identification of MARs near translocation sites may provide information that could be used to improve diagnosis and detection of these key rearrangements, or in the discovery of drug targets for cancers associated with chromosomal translocations. Acknowledgment: This research was supported in part through the MBRS SCORE Program and a Research Infrastructure in Minority Institutions from the National Institutes of Health.

**Genomic Imbalances in Schwann Cells of Neuroblastic Tumors.** *G. Kim<sup>1</sup>, S. Park<sup>2</sup>, S. Park<sup>3</sup>, C. Kim<sup>4</sup>.* 1) Perinatology Research Branch, National Institute of Child Health and Human Development, Department of Obstetrics and Gynecology, Wayne State University/Hutzel Hospital, MI, USA; 2) Department of Anatomy, Ehwa womans University College of Medicine, Seoul, Korea; 3) Institute of Human Genetics, Brain Korea 21 Biomedical Sciences, Korea University College of Medicine, Seoul, Korea; 4) Department of Pathology, Seoul National University College of Medicine, Seoul, Korea.

Neuroblastic tumors are characterized by their potential for differentiation *in vivo* and *in vitro*. *In vivo* differentiation into neuroblastic/ganglionic cells and Schwannian stromal cells are frequently encountered and it has been believed that neural crest-derived sympathetic precursor cells undergo differentiation into both lineages. However, some of the recent studies raised a possibility that Schwannian stromal cells are nonneoplastic cells, which have been recruited from surrounding milieu. We assessed this issue by comparative screening of genetic constitution of neuroblastic/ganglionic cells and Schwannian stromal cells using degenerated oligonucleotide primed-PCR comparative genomic hybridization. A total of five cases of ganglioneuroblastoma and a case of ganglioneuroma were analyzed. Both cell types had frequent gains and losses showing that Schwannian cells are also neoplastic. Schwannian stromal cells shared some of the genetic changes with neuroblastic/ganglionic cells in same tumors. We additionally screened lymph node metastasis of neuroblastic tumors and could easily demonstrate S-100 positive Schwannian cells in metastatic lymph nodes. Genomic imbalances and metastatic potential of Schwannian stromal cells clearly indicate that Schwannian stromal cells are neoplastic. In the context that Schwann cells interact with neuronal cells via several signals, biological pathways driving the neoplastic cells to differentiate into Schwannian stromal cells are worth to be further investigated.

**Genomic imbalance changes associated with malignant potential in gastrointestinal stromal tumors.** *Y. Chen*<sup>1</sup>, *C.C. Tzeng*<sup>2</sup>, *C.P. Liou*<sup>2</sup>, *M.Y. Chang*<sup>2</sup>, *C.F. Li*<sup>2</sup>, *C.N. Lin*<sup>2</sup>. 1) Department of Surgery, Chi-Mei Foundation Hospital, Tainan, Taiwan, Republic of China; 2) Department of Pathology, Chi-Mei Foundation Hospital, Tainan, Taiwan, Republic of China.

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors of gastrointestinal tract. Current criteria for the diagnosis of malignant GISTs do not always reliably predict patient outcome. In order to search for genetic markers with prognostic potential, chromosomal imbalance changes in 28 GISTs were analyzed using comparative genomic hybridization and correlated with clinicopathological characteristics. Except a small rectal tumor, all other GISTs, including 14 from stomach, 11 from small intestine, one from esophagus, and one from rectum, exhibited chromosomal alternations. Losses were more common than gains. The median number of chromosomal changes in high-risk GIST was significantly higher than in low-risk GIST (5.602.59 vs. 3.382.55), especially for the changes of loss (4.601.84 vs. 2.632.13). Loss of 14q was the most common change, not only in low-risk GISTs (70.6%, 12/17) but also in high-risk GISTs (54.5%, 6/11). Several other additional changes detected in more than 25% of high-risk GISTs were losses of 1p, 10q, 13q, 15q, 18q, and 22q and gains of 5p, 12q, 17q, and 20q. Among these changes, more frequently detected in high-risk GISTs than in low-risk GISTs were losses of 13q (5/11 vs. 1/17,  $P = 0.044$ ) and 22q (7/11 vs. 3/17,  $P = 0.038$ ). Finally, the most striking finding was loss of 10q, with minimal overlapping on q11-q22, which was identified only in two metastatic small intestinal GISTs and one esophageal GIST. Our finding, together with results reported by previous studies, first indicated that loss of 10q, probably on q11-q22, seems to be chromosomal loci potentially harboring tumor suppressor gene(s), which may relate to early recurrence and/or metastasis of GISTs during malignant transformation.

**Some three way translocations arise in two steps.** *H.A. Aviv, A. Gonzalez, I. Maxwell, P. DeAngelo, S. Szardenings.* Pediatrics/Genetics, MSB F669, UMDNJ-New Jersey Medical School, Newark, NJ.

The 9;22 translocation in chronic myeloid leukemia (CML) can be a simple reciprocal translocation or a three way translocation involving an intermediate chromosome. The latter also results in Philadelphia (Ph) chromosome positive classical CML. Patients with both types of translocations usually have the same prognosis. The mechanism by which three way translocations are formed is unknown, but it is assumed that all three chromosomes undergo breakage and rearrangement simultaneously. We present a patient with acute lymphoblastic leukemia (ALL) and the classical 9;22 translocation at diagnosis. At relapse, the patient had a three way translocation involving chromosomes 9, 22 and 6. The apparent three way translocation thus arose from a second translocation involving the derivative chromosome 9 and chromosome 6. The pattern of 2 fusion signals obtained with BCR/ABL dual fusion probes also supports this conclusion. The mechanism of two consecutive translocations masquerading as a three way translocation may be more common in hematological malignancies due to increased chromosomal instability. Patients with "pseudo" three way translocations may have a more advanced disease and worse prognosis than patients with "classical" three way translocations. The use of dual fusion probes, when available, helps to distinguish between the two types of translocations. The use of dual fusion probes, when available, helps to distinguish between the two types of translocations. Complex constitutional translocations may also be formed by the same mechanism. We are currently investigating the origin of other three way translocations, both acquired and constitutional.

**Cytogenetic characterization of thyroid tumors and cell lines using comparative genomic hybridization, spectral karyotyping and G-banding.** *T. Foukakis*<sup>1,2,3</sup>, *S.R. Thoppe*<sup>1</sup>, *S. Lagercrantz*<sup>1</sup>, *T. Dwight*<sup>1,2</sup>, *A. Svensson*<sup>3</sup>, *W.O. Lui*<sup>1</sup>, *G. Wallin*<sup>3</sup>, *J. Zedenius*<sup>2</sup>, *C. Larsson*<sup>1</sup>. 1) Endocrine Tumor Unit, Department of Molecular Medicine, Karolinska Hospital CMM L8:01, Stockholm, Sweden; 2) Centre for Metabolism & Endocrinology, Department of Surgery, Karolinska Institutet at Huddinge University Hospital, Stockholm, Sweden; 3) Department of Surgery, Karolinska Hospital P9:03, Stockholm, Sweden.

To further delineate the role of chromosomal aberrations in thyroid tumors, we performed karyotypic analyses of thyroid cell lines and primary tumors. We cytogenetically characterized five established thyroid cancer cell lines (MRO, DRO-90, ARO-81, CGTH W-1, CGTH W-3) by comparative genomic hybridization (CGH), spectral karyotyping (SKY) and regular G-banding. By CGH, the most common gains were detected at chromosomes 1,5,7,10,11,17,19,20 and 21, whereas losses were most common at chromosomes 4, 5, 6, 9, 10 and 18. SKY, in combination with G-banding revealed karyotypic abnormalities, structural and numerical, in all five cell lines. Moreover, we karyotyped 16 primary thyroid tumors using SKY. Three of the 11 thyroid carcinomas studied exhibited abnormal karyotypes. In case T1, a follicular thyroid carcinoma, we observed translocations t(2;10), t(2;5) and losses of chromosomes 10 and 22. In tumor T2, a papillary thyroid carcinoma (PTC), we observed a balanced translocation t(3;15), while T3, a case of metastatic PTC, carried several clonal translocations involving chromosomes 1,2,3,5,14,15,16,17 and 19. Numerical aberrations were observed in two of the five follicular adenomas we analyzed. CGH was later performed for the cases harboring abnormal karyotypes, and the copy number changes correlated well with the karyotypic findings. Our results provide evidence of chromosomal regions frequently involved in thyroid tumorigenesis, while further characterization of the observed translocations may lead to the identification of novel fusion oncogenes for thyroid cancer.

**Molecular cytogenetic characterization of a case of Müllerian Adenosarcoma.** *E. Drozd-Borysiuk<sup>1</sup>, B. Issa<sup>1</sup>, B. Hong<sup>1</sup>, J. Stratton<sup>1</sup>, A. Brothman<sup>1</sup>, C. Coffin<sup>2</sup>, K. Albritton<sup>3</sup>, Z. Chen<sup>1</sup>.* 1) Pediatrics/Cytogenetics, University of Utah, Salt Lake City, UT; 2) Department of Pathology, University of Utah, Salt Lake City, UT; 3) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT.

A variety of tumors of Müllerian origin, composed of both epithelial and mesenchymal elements, have been described in the literature. These tumors range from benign entities, such as a papillary adenofibroma, through the locally aggressive adenosarcoma to the fully malignant forms of malignant mixed Müllerian tumors. Müllerian adenosarcoma is a distinctive type of mixed Müllerian tumor of the female genital tract. So far no cytogenetic data have been documented on Müllerian adenosarcoma in the literature. We report here the chromosomal findings of a Müllerian adenosarcoma in a 15-year-old female. Cytogenetic and molecular cytogenetic analysis revealed a complex karyotype involving chromosomes 2, 8, 10, 13, 19, and 21. These numerical and structural abnormalities may be of etiologic significance. This report may highlight the potential value of molecular cytogenetic analysis in differential diagnosis of Müllerian tumors. More cases are warranted to further genetically characterize this type of neoplasm.

**Does FISH improve the cytology detection in urothelial bladder carcinoma? A Brazilian experience. G.J.F.**

*Gattas*<sup>1,2</sup>, *A.C. Bomfim*<sup>3</sup>, *M. Srougi*<sup>3</sup>, *K.R.M. Leite*<sup>2</sup>, *L.H. Camara-Lopes*<sup>2</sup>. 1) Dept of Legal Med, Bioethics and Occup Health, Medical School, São Paulo University, São Paulo, Brazil; 2) Molecular and Surgical Pathology Laboratory of Sirio Libanês Hospital, São Paulo, SP, Brazil; 3) Division of Urology, Federal University of São Paulo, São Paulo, SP, Brazil.

The urothelial carcinoma (UC) is the most common malignancy of the urinary tract. Bladder tumors are grossly staged by superficial (pTa-T1 stages) or invasive (pT2-T4 stages) related to the bladder wall invasion. We prospectively evaluated the sensitivity and specificity of cytology and FISH for the detection of UC in voided urine specimens of three groups: group 1 with 53 UC patients (33 men: 72,87 yo; 20 women: 67,414 yo) with tumor identification by cytology and histopathology (17 pTa, 17 pT1, 9 pT2, 7 pT3, 2 pT4); group 2, 23 individuals (mean age 6510 yo) with previous history of UC; group 3, 42 patients (mean age 6712 yo) with prostate hyperplasia. For FISH evaluation we used UroVysion Multi-color Probe (Vysis) and the positive criteria recommended. For the group 1 the cytology and FISH was positive for 35/53 (66%) and 38/49 (77,5%) and the analysis was not possible in 4 FISH cases; group 2 cytology and FISH was positive for 6/24 (25%) and 9/24 (37,5%); group 3 the cytology and FISH was positive for 11/42 (26%) and 7/39 (17,9%) with 3 FISH cases not analysed. Only the FISH was 100% positive for tumors from pT1-pT4 stages (32/32). For both techniques the pTa stage tumors were poorly identified (35%). The FISH sensitivity was higher (77%) compared with cytology (66%) although the specificity was very similar for both (75% FISH; 74% cytology). In superficial tumors the recurrence risk (70-90% in ten years) and the progression to invasive tumor (pTa: 2-4%; pT1:20-30%) are very important. If we considered that in the pT1 stage tumors the patients treated by endoscopy resection are at risk of 20-30% for progression to pT2-T4 stages, with poor prognosis, the FISH was more informative than cytology. We are increasing the sample to better understand the contribution of each test in urine UC diagnostic. This study was supported by FAPESP.

**Molecular and cytogenetic characterization of a novel translocation 4;22 involving BCR and PDGFRA in a patient with chronic myelogenous leukemia (CML).** *B.K. Goodman<sup>1</sup>, A.M. Safley<sup>2</sup>, S. Sebastian<sup>1</sup>, C.A. Tirado<sup>1</sup>, T. Collins<sup>3</sup>, T.T. Stenzel<sup>4</sup>, J.Z. Gong<sup>1</sup>.* 1) Department of Pathology; 2) School of Medicine; 3) Department of Medicine, Duke University Medical Center, Durham, NC; 4) Vysis, Inc., Downers Grove, IL.

We report a case of Philadelphia chromosome-negative chronic myelogenous leukemia (CML) with translocation (4;22)(q12;q11.2) juxtaposing the breakpoint cluster region (BCR) and platelet-derived growth factor-alpha (PDGFRA) loci. The patient, a 57 year-old male with a history of stage IV diffuse large B-cell lymphoma status post six cycles of combination chemotherapy in 1999, presented in August 2002 with enlarged lymph nodes, anemia and marked leukocytosis, consistent with a myeloproliferative disorder (MPD). A bone marrow biopsy showed granulocytic hyperplasia, neutrophilia and mild eosinophilia. Initial evaluation using interphase FISH for BCR-ABL, to rule out the translocation 9;22, showed a variant signal pattern consistent with rearrangement of BCR at 22q11.2, but not ABL at 9q34. Analysis of patient cDNA by reverse transcription polymerase chain reaction (RT-PCR) for the BCR-ABL transcript was negative. Routine G-band chromosome analysis of the patient's bone marrow showed, instead, an abnormal clone with rearrangement of chromosomes 4 and 22. We performed RT-PCR using primers for BCR and a candidate gene at 4q12, and sequenced the product, demonstrating an in-frame 5'-BCR/3'-PDGFRA fusion transcript that includes a short intronic region. PDGFRA encodes a receptor tyrosine kinase and shares structural and organizational homology with the c-kit and colony stimulation factor-1 receptor genes. While the incidence of MPD involving translocations of PDGFRB has been well established, to our knowledge there has been only one previous report describing a BCR-PDGFRA fusion gene, in two patients diagnosed with atypical CML. Here we present molecular and cytogenetic characterization of BCR-PDGFRA-positive MPD in a patient who is showing regression of leukocytosis following treatment with imatinib mesylate, which targets the tyrosine kinase domain of the ABL locus.

**Analysis of 25 cases of gastric adenocarcinoma with CGH.** *S. Cheng*<sup>1</sup>, *C. Wang*<sup>1</sup>, *W. Chuang*<sup>2</sup>, *T. Hwang*<sup>3</sup>. 1) Dept Anatomy, Chang Gung Univ, Col Medicine, Kweishan, Taoyuan, Taiwan; 2) Dept Pathology, Chang Gung Memorial Hospital, Kweishan, Taoyuan, Taiwan; 3) Dept Surgery, Chang Gung Memorial Hospital, Taipei, Taiwan.

Gastric adenocarcinoma specimens were collected from the tissue bank in the Department of General Surgery, Chang Gung Memorial Hospital, Linkou, Taiwan. With cryosection and hematoxylin and eosin staining the tumor tissues were identified, and the specimens with primarily tumor tissue were selected. The genomic DNAs were extracted from selected tissues for labeling with biotin-16-dUTP by nick translation. The DNAs from normal male and female are served as control, and are labeled with digoxigenin-11-dUTP. The technique comparative genomic hybridization (CGH) was used for examining the copy number change of chromosomes of individual gastric tumor cases. Among 25 cases examined 14 cases are of tubular type, and 11 cases are signet-ring cell type according to WHO classification. Basing on Lauren classification 14 cases are of intestinal type. The 14 cases are same as the 14 cases of WHO classification. The other 11 cases are of diffuse type. The CGH analyses were completed with Quips software (Applied Imaging). Among the 14 cases of tubular type 50% have a gain at 1q, 43% at 3q, and 36% at 4q; while 29% have a loss at 9p. Among the 11 cases of signet-ring cell type 55% have a gain at 4q and 5q, 45% at 6p and 8q, 36% at 3q, 5p, and 8p, and 27% at 1q, 20p, and 20q; while 36% have a loss at 9q, 16q, 21q, and 22q, 27% at 1q, 13p, 16p, 17q, and 22p.

**Homozygous deletion of p16 gene in childhood acute lymphoblastic leukemia : clinical outcome and prognostic significance.** *S. Barrette*<sup>1</sup>, *S.K. Murthy*<sup>2</sup>. 1) Departments of Haematology and Oncology, Sainte Justine Hospital, Montreal, PQ, Canada; 2) Department of Pathology, Cytogenetics Laboratory, Sainte Justine Hospital, Montreal, PQ, Canada.

Cytogenetic abnormalities involving chromosomal band 9p21-22 has been reported in 7% to 13% of acute lymphoblastic leukemia (ALL). Homozygous deletion of both p16 and p15 genes occur in at least 60% of T- and 20% of B-cell lineage cases, and is the major means of gene inactivation in childhood ALL. It is mainly associated with higher relapse rates and poor outcome. We present here a case of childhood B-ALL with homozygous deletion of p16. At age 3 1/2 years, she presented with WBC count of 27,000/uL, CNS negative, no mediastinal mass, and was treated according to the standard risk protocol of DFCI 95-001 (Dana Farber Cancer Institute), with no cranial irradiation. Cytogenetic analysis at initial diagnosis showed a normal karyotype. RT-PCR for t(9;22), t(12;21), t(4;11), t(1;19) and FISH for 11q23 were negative. She responded well to the therapy which ended in Sept 2001. In Nov 2001 she presented with facial palsy and relapse in both bone marrow and CNS was diagnosed. Chromosome analysis showed presence of del(9p) in 7 out of 24 cells. She was then treated according to the high-risk arm of DFCI 2000-01. Remission was attained within a month of treatment and was consolidated with a fully matched unrelated bone marrow transplantation. She did well for 124 days post transplant, but again had a marrow and CNS relapse. FISH analysis with LSI p16/CEP9 probe (Vysis) showed homozygous deletion of p16 in 61% cells and heterozygous deletion in 13% cells. The patient died in Sept 2002 at age 6. The second case is a 23 months old female with pre B-cell ALL. Her karyotype at diagnosis was 45,XX,i(9)(q10),der(20) and FISH with p16 probe showed homozygous loss in 80% of cells. She is still under treatment. Subtle cytogenetic rearrangements involving p16 may be missed by routine cytogenetics and thus a careful molecular cytogenetic analysis is recommended to identify this subgroup of standard risk patients with increased risk of treatment failure, and poor outcome.

**Genetic abnormalities in human Langerhans Cell Histiocytosis derived cell line (DOR-1) detected by Comparative Genomic Hybridization.** *J. GOGUSEV<sup>1</sup>, L. TELVI<sup>2</sup>, I. MURAKAMI<sup>1</sup>, C. GLORION<sup>3</sup>, F. JAUBERT<sup>4</sup>.* 1) INSERM U507, Necker Hosp, Paris; 2) Cytogenetics Laboratory, Paris; 3) Orthopaedic Surgery Department, Paris; 4) Pathology Department, Paris, France.

Langerhans cell histiocytosis (LCH) is a disease characterized by the presence of destructive granulomas containing CD1a+ Langerhans-like dendritic cells admixed with lymphoid and eosinophilic cells. Recent molecular genetic studies indicated monoclonal cell expansion and chromosomal abnormalities in both unifocal and disseminated LCH. Very recently, we established in our laboratory a LCH-derived cell line, called DOR-1. This cell line was established from an unifocal lytic lesion of bone from a three years old girl. DOR-1 cells grow as a flat adherent stromal-like cells. On R-banding a notably complex karyotype was observed with numerous aberrant chromosomes including a large dicentric marker present in more than 10% of cell metaphases. In addition, a structural rearrangement t(9;17) was found in approximately 20% of the metaphase spreads indicating clonal abnormality. The t(9;17) was confirmed by dual-color FISH using specific whole chromosomes painting probes specific for chromosomes 9 & 17 and by CGH technique. Repeated CGH experiments demonstrated genomic aberrations in DOR-1 cells appearing as a DNA copy number losses located on chromosomes 1p, 5p, 7p, 9q, 13q, 16p, 17p & 18. DNA copy number gain was observed on chromosomes 2 & 12. For comparison, CGH analysis was applied to the identification of genomic changes in a histologically defined area from the original LCH lesion. DNA copy number losses were detected on chromosomes 1p, 5, 6, 7, 9, 16, 17 & 22q. Gain of DNA copy number was observed on chromosome 2q, 4q & 12. These findings indicate that DOR-1 cells exhibit complex molecular cytogenetics anomalies and share certain molecular features with the original LCH tissue. The structural t(9;17) and the large dicentric chromosome might be serve as a remarkable new chromosomal markers in early diagnosis and treatment of patients.

**Cellular Leiomyoma: A Genetically Distinct Entity Among Benign Uterine Tumors.** *N. Christacos*<sup>1,3</sup>, *P. Dal Cin*<sup>2,3</sup>, *C.C. Morton*<sup>1,2,3</sup>, *B.J. Quade*<sup>2,3</sup>. 1) Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Womens Hospital, Boston, MA; 2) Department of Pathology, Brigham and Womens Hospital, Boston, MA; 3) Harvard Medical School, Boston, MA.

Uterine leiomyomata (UL) are the most common pelvic tumors in women. These tumors occur in nearly 77% of women of reproductive age. Cytogenetic analysis of UL show that about 40% of these tumors have simple, clonal chromosomal rearrangements. In contrast, leiomyosarcomas (LMS), typically have complex numerical and structural abnormalities. Currently, little is known about the cytogenetics and molecular genetics of the histologic variants of UL. From the subset of karyotypically abnormal UL out of ~ 1000 karyotyped tumors, we identified a group of 8 cases exhibiting near diploid karyotypes with loss of almost the entire short (p) arm of chromosome 1 (del(1)(p11p36)). Loss of 1p was frequently associated with other aberrations. The histological diagnosis was available in 5 of the 8 tumors. Four tumors were diagnosed as cellular UL; and one tumor displayed both hypercellularity and nuclear atypia. LOH for chromosomal regions 1p36.2 and 1p11.1 using PCR on archival DNA from 5 UL with the histological diagnosis of cellular or atypical leiomyomata and matched myometrium was performed. The cytogenetic analysis of these archival UL was unknown. We found that UL from 2 out of 5 individuals demonstrated LOH for either the majority of the short arm of chromosome 1 (del(1)(p11p36)) or part of the short arm of chromosome 1 (del(1)(p11)). RNA from selected tumors was profiled using Affymetrix GeneChips and those profiles were compared to previously reported expression profiles of myometrium, UL, and uterine LMS using 153 probe sets. The transcriptional profile of two tumors with chromosome 1 abnormalities were more similar to those of LMS than to profiles of myometrium and UL as determined by hierarchical cluster analysis. Loss of 1p in UL appears to define a subgroup of tumors distinct from those previously recognized. Further, 1p- appears to be associated with a specific histologic phenotype. The similarity between the transcriptional profiles of LMS and UL with 1p- supports a common pathogenetic mechanism.

**Clonal evolution in a case of acute promyelocytic leukemia.** *S.R.S. Gottesman<sup>1</sup>, A. Babu<sup>2</sup>, R. Zeng<sup>2</sup>, V.*

*Mizhiritskaya<sup>2</sup>, M.J. Macera<sup>2</sup>.* 1) Department of Pathology, SUNY Downstate Medical Center, Brooklyn, NY; 2) Division of Molecular Medicine and Genetics, Department of Medicine, Wyckoff Heights Medical Center, Brooklyn, NY.

A 69 year old female, at preadmission for insertion of a cardiac pacemaker, was found to be severely neutropenic with normal red blood cell counts and normal platelets. A repeat test, one month later, showed that she had a neutrophil count of 179/L with normal platelets (166K/L), decreased lymphocytes (1024/L) and slightly decreased red blood cells ( $3.7 \times 10^6/\text{fL}$ ). The patient received granulocyte-colony stimulating factor after which she raised her white blood cell count only minimally but increased her percentage of mature neutrophils. Within two weeks, her platelets dropped to a critical level of 17K/L and a bone marrow was performed. Chromosome analysis of the bone marrow revealed three separate cell lines, 46,XX, der(5)t(5;12)(p13;q15), ?del(11)(p11.2p12),der(12)(1qter1q25::12p1312q15::5p135pter),t(15;17)(q22;q21) [30]/46,XX,t(5;12)(p13;q15),?del(11)(p11.2p12),t(15;17)(q22;q21),add(20)(p12)[11]/46,XX [9]. The presence of the t(15;17) was confirmed by FISH analysis. The cytogenetic hallmark of acute promyelocytic leukemia (APL, M3) is the presence of a translocation between chromosomes 15 and 17, as the t(15;17) is present in close to 100% of the reported cases and therefore is diagnostic for APL (AML-M3). Unlike other leukemias, the t(15;17) is generally seen as a sole cytogenetic abnormality. Clonal evolution typically proceeds with minimal abnormalities and then additional abnormalities are acquired as the disease progresses; here both cell lines have three common abnormalities, the t(15;17), t(5;12) and the del (11), with an acquired unique abnormality. This case is very interesting because of the complex abnormalities associated with the t(15;17) and its rather rapid clonal evolution as evident from two distinct sidelines (clones). It is essential to follow up such rare cases for they may provide information that is helpful towards understanding the course of the disease and its therapeutic respons.

**Incidence of mono- and bi-allelic deletions involving 13q14 in B-cell chronic lymphocytic leukemia (B-CLL) as detected by fluorescence in situ hybridization (FISH).** *A.W. Block<sup>1</sup>, J.M. Panasiwicz<sup>1</sup>, K.C. Miller<sup>2</sup>, Z.P. Bernstein<sup>2</sup>, A. Chanan-Khan<sup>2</sup>.* 1) Clinical Cytogenetics Lab, Roswell Park Cancer Inst, Buffalo, NY; 2) Department of Medicine, Roswell Park Cancer Inst, Buffalo, NY.

B-CLL, the most common leukemia in adults, is a malignant disorder characterized by progressive accumulation of mature appearing functionally compromised B-lymphocytes. Conventional cytogenetic (CC) and FISH-based studies have identified recurrent numerical and structural chromosome abnormalities with prognostic significance in B-CLL. Patients with 13q14 deletion, the most common aberration, are reported to have favorable prognosis. Using a FISH panel that identified specific numerical and structural abnormalities (+12, del(13q), del(11q), del(17p), 14q32 abn), we studied peripheral blood and bone marrow from 55 unselected B-CLL patients (pts) to investigate the incidence and clinical relevance of mono- and bi-allelic deletions involving the 13q14 region. The Vysis, Inc. LSI 13 (containing RB1) and D13S25 (1.6 cM distal to RB1) probes were co-hybridized to determine copy number and estimate deletion size. 24/55 pts (15 males, 9 females; median age 61, range 38-85 yrs) had either a mono- (n=14) or bi-allelic (n=10) deletion of 13q14. Range in deletion size was noted with mono-allelic deletion of D13S25 and mono-allelic deletion of both RB1 and D13S25 in 9/24 and 5/24 pts, respectively. 10/24 pts showed bi-allelic deletion of D13S25 with 4/10 also having mono-allelic deletion of RB1. Cell populations were heterogeneous with losses of 1-2 copies of D13S25 in 7/24 pts. Two or more concurrent aberrations were seen in 7/24 pts with 6/7 pts having del(11q); these pts had chemotherapy refractory advanced stage disease and poor clinical outcome. A model has been proposed for B-CLL pathogenesis, suggesting deletion of putative enhancer sequences and/or genes resulting in inactivation of tumor suppression by haploinsufficiency. Alternatively, inherent instability in this region may lead to the interstitial deletions of 13q14 that are also seen in other neoplasms including multiple myeloma, mantle cell and peripheral T cell lymphoma, prostate cancer, and squamous cell carcinoma of the head and neck.

**Ubiquinol cytochrome-c reductase (UQCRFS1) gene amplification in primary breast cancer core biopsy samples.**

*Y. Ohashi<sup>1</sup>, R. Young<sup>1</sup>, S. Kaneko<sup>2</sup>, T. Copples<sup>3</sup>.* 1) Dept OB/GYN, Univ South Carolina, Columbia, SC; 2) Dept OB/GYN, Nagoya City Univ, Nagoya, Japan; 3) Dept Radiology, Palmetto Health Alliance, Columbia, SC.

Ubiquinol cytochrome-c reductase (UQCRFS1), otherwise known as the Rieske Fe-S protein, is a key subunit of the cytochrome bc1 complex (complex3) in the mitochondria. This protein is important in redox reactions. Although UQCRFS1 is used primarily in mitochondria, the gene itself is located in the nuclear genome (19q12). We recently reported amplification of UQCRFS1 gene in ovarian carcinoma using FISH probes. Including our previous study on ovarian carcinoma, there have been just two articles reporting UQCRFS1 amplification associated with malignancy. However, there has thus far been no report of UQCRFS1 gene amplification associated with breast cancer. The purpose of this study is to evaluate gene copy number of UQCRFS1 gene in primary breast cancers.

Samples were obtained from image-guided core-needle biopsies in forty patients with non-treated breast cancers. Twenty-nine cases were histopathologically classified as invasive ductal carcinoma, 1 as ductal carcinoma in situ, 9 as invasive lobular carcinoma and 1 as invasive mixed carcinoma. To examine UQCRFS1 gene copy number, we employed FISH using BACs RP11-46I12 (19q12-19q13) which harbors UQCRFS1 gene, RP11-110J19 (19p11-19q11) which overlap to RP11-46I12 and CA125 (19p13.2) as a control gene. A sample with a copy number ratio (UQCRFS1/CA125) greater than 2.0 was considered to be amplified. The data were evaluated blindly of histopathological factors. UQCRFS1 copy number per cell ranged from 1.9 to 18.6, the mean was 3.3. CA-125 copy number per cell ranged from 1.0 to 3.6, the mean was 2.3. Amplification of UQCRFS1 gene was found in five of 39 specimens (12.8%). All of these amplified cases showed between 4.0 and 18.6 signals per cell. The specimens with amplified UQCRFS1 gene were significantly associated with high grade of cancer cells ( $p=0.005$ ). Our results suggest that the UQCRFS1 gene appears to be involved in development of more aggressive phenotype of breast cancer.

**Do cytogenetic abnormalities precede morphological abnormalities in developing malignant conditions? J.K.**

*Northup<sup>1</sup>, Y. Ge<sup>1</sup>, S. Gadre<sup>1</sup>, L.H. Lockhart<sup>2</sup>, G.V.N. Velagaleti<sup>1,2</sup>.* 1) Department of Pathology; 2) Department of Pediatrics, University of Texas Medical Br, Galveston, TX.

Cytogenetic evaluation of bone marrow and neoplastic tissues plays a critical role in determining patient management and prognosis. Here we highlight two cases in which the cytogenetic studies challenge the common practice of using hematological and morphological changes as key factors in malignant disease management. The first case is that of a lymph node sample from a 40-year-old male with a history of non-Hodgkins lymphoma sent to determine if the lymphoma had progressed to high-grade. Cytogenetic studies from bone marrow showed multiple clonal abnormalities, most notably a der(18) from a t(14;18) which is associated with high-grade NHL. Results of hematological studies of the same tissue showed no evidence of transformation to high-grade (less than 15% blasts with rare mitotic figures). In spite of two cycles of chemotherapy with fludarabine, the patient has not shown any improvement clinically. The non-responsiveness of this patient to traditional chemotherapy suggests possible progression to high-grade lymphoma as suggested by cytogenetic analysis. The second case is of a patient with a history of HIV and blastic NK leukemia/lymphoma. Hematological studies of ascitic fluid classified the patient as having primary effusion lymphoma (PEL). Hematological studies on bone marrow from this patient were normal with no evidence of malignancy. Cytogenetic evaluation of the bone marrow showed multiple clonal abnormalities including a t(8;14). This rearrangement is commonly associated with Burkitts lymphoma (BL). To our knowledge, this is the first case wherein a morphologically normal bone marrow showed presence of clonal abnormalities consistent with BL or PEL. After two cycles of CHOP chemotherapy, the patients general condition and ascitis improved and she was discharged. These studies clearly demonstrate that genetic changes often precede morphological change in a developing malignant condition. Therefore, the critical information needed to manage patient care of malignant disorders may be incomplete or inaccurate if cytogenetic evaluation is overlooked.

**Cytogenetic and molecular cytogenetic evidence of distinct cell populations with bcr/abl fusion involving M-bcr and m-bcr breakpoints in a patient with chronic myeloid leukemia.** *A.S. Kulharya<sup>1,2</sup>, K.L. Satya-Prakash<sup>1,2</sup>, Y. Nalamolu<sup>2</sup>, F. Mazella<sup>2</sup>, J. Hiemenz<sup>2</sup>.* 1) Dept Pediatrics, Medical Col Georgia, Augusta, GA; 2) Dept Pathology and Medicine, Medical Col Georgia, Augusta, GA.

In 95% of cases of chronic myeloid leukemia (CML), the BCR-ABL fusion involves a break within the major breakpoint cluster region (M-bcr) resulting in a p210 fusion protein. Rare cases of CML have been described involving a break within the minor breakpoint cluster region (m-bcr) resulting in a p190 fusion protein. In several studies co-expression of p190 and p210 has been shown in CML patients in chronic phase. The co-expression of major and minor transcripts has been demonstrated only by RT-PCR analysis. We present a 40-year old leukemia patient with distinct cell populations of BCR/ABL fusion involving minor and major breakpoints in the BCR gene. Cytogenetic analysis of bone marrow showed two distinct abnormal clones, one with classic Ph chromosome and another with translocation of BCR gene to a rearranged chromosome 9. The karyotype is: 46,XY,t(9;22)(q34;q11.2)[2]/46,XY,der(9)inv(9)(p13q34)t(9;22)(q34;q11.2)[18]. FISH was performed using a BCR/ABL fusion probe that detects M-bcr and m-bcr breakpoints within the BCR gene. Eighty percent of the cells analyzed are consistent with a fusion at the minor breakpoint and 15% cells are consistent with a fusion of bcr and abl at the Major breakpoint. The remaining 5% cells do not show any bcr/abl fusion. It is concluded from these observations that the translocation involving chromosome 9 with an inversion represents the clone with the m-bcr breakpoint while the smaller clone with an apparent classic Ph chromosome represents a break at the M-bcr in the BCR gene. Alternative splicing of the BCR gene is thought to be responsible for the co-expression of M-bcr and m-bcr transcripts as two distinct cell populations have not been shown previously. Our patient provides physical evidence of two distinct cell populations that individually involve the M-bcr and m-bcr breakpoints in the fusion of BCR and ABL gene. These two cell populations indicate that independent clonal events are responsible for co-expression M-bcr and m-bcr products.

**Chromosome Analyses of 16 Cases of Wilms Tumor: Different Pattern in Unfavorable Histology.** A. Mohamed<sup>1</sup>, E. Peres<sup>2</sup>, B. Cushing<sup>2</sup>, S. Abella<sup>2</sup>, S. Savasan<sup>2</sup>. 1) Dept Pathology, Cancer Cytogenetics, Wayne State Univ, Detroit, MI; 2) Division of Pediatric Hematology/Oncology, Childrens Hospital of Michigan, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, 48201.

Wilms tumor is the most common malignant tumor of the kidney in the pediatric population, accounting for 98% of childhood renal cancers. The incidence of Wilms tumor is 1 per 10,000 children, with the majority of cases being diagnosed before the age of 6 years. Cytogenetic analyses of 16 cases of Wilms tumor with abnormal karyotype were reviewed. Fifteen cases had unilateral tumors and one was bilateral. Three tumors exhibited the unfavorable histology whereas the remaining tumors were categorized into the favorable histology group. Of the 17 tumors with abnormal clonal aberrations: nine tumors were hyperdiploid (53%), seven had pseudodiploid karyotypes (41%), and one was hypodiploid (6%). The most common numerical aberrations in descending order were gain of chromosomes 12, 8, 6, and loss of chromosome 16. Structural rearrangements mostly involved chromosome 1 followed by chromosomes 7, 14, and 17. Clustering of breaks around 1p22-31pter resulting in partial loss of 1p was the most frequent structural aberration. Additionally, i(7q) was observed as a sole abnormality in two tumors and a 7p translocation in two other tumors. Other interesting recurrent abnormality was a partial deletion of 14q, seen in three tumors and complete loss of 14 in one tumor. All three Wilms tumors with unfavorable histology had abnormalities of 17p resulting in P53 gene deletion. These findings provide further support for the importance of gains of chromosomes 12, 8, and 6, and loss of 1p in the development of Wilms tumor. The results also support the association of unfavorable histology Wilms tumors with P53 deletion. The nonrandom losses of 16/16q, 7p, and 14q may represent the importance of genomic imbalance in the pathogenetic consequence and progression of Wilms tumor.

**Karyotypic complexity of the NCI-60 drug-screening panel.** *I. Kirsch<sup>1</sup>, A. Roschke<sup>1</sup>, G. Tonon<sup>1</sup>, K. Gehlhaus<sup>1</sup>, N. Mctyre<sup>1</sup>, K. Bussey<sup>2</sup>, S. Lababidi<sup>2</sup>, D. Scudiero<sup>3</sup>, J. Weinstein<sup>2</sup>.* 1) Genetics Branch, National Cancer Institute, Bethesda, MD; 2) Laboratory of Molecular Pharmacology, National Cancer Institute, Bethesda, MD; 3) SAIC-Frederick Cancer Research and Development Center, Frederick, MD.

We used SKY to provide a detailed analysis of karyotypic aberrations in the diverse group of cancer cell lines established by the National Cancer Institute for the purpose of anti-cancer drug discovery. Along with the karyotypic description of these cell lines we defined and studied karyotypic complexity and heterogeneity (metaphase-to-metaphase variations) based on three separate components of genomic anatomy: 1) ploidy, 2) numerical changes, and 3) structural rearrangements. A wide variation in these parameters was evident in these cell lines, and different association patterns between them were revealed. Analysis of the breakpoints and other specific features of chromosomal changes across the entire set of cell lines or within particular lineages, pointed to a striking lability of centromeric regions that distinguishes the epithelial tumor cell lines. We have also found that balanced translocations are as frequent in absolute number within the cell lines derived from solid as from hematopoietic tumors. Important similarities were noticed between karyotypic changes in cancer cell lines and those seen in primary tumors. This dataset offers insights into the causes and consequences of the destabilizing events and chromosomal instability that may occur during tumor development and progression. It also provides a foundation for investigating associations between structural genome anatomy and cancer molecular markers and targets, gene expression, gene dosage, and resistance or sensitivity to tens of thousands of molecular compounds.

**Emergence of deletion on derivative chromosome 9 in CML with t(9;22) during disease progression: a new clone vs. clonal evolution.** *N. Mitter*<sup>1</sup>, *P. Berry*<sup>1</sup>, *P. Patel*<sup>2</sup>, *M. Donskoy*<sup>1</sup>, *J. Lovell*<sup>1</sup>, *M. Graham*<sup>1</sup>. 1) Dianon Systems (Lab Corp, Inc.), Stratford, CT; 2) Stuart Oncology Associates, Stuart, FL.

Many patients with CML show acquired deletions 5 of ABL and 3 of BCR on the derivative chromosome 9, associated with a reduced long-term survival. Empirical observations suggested that such deletions occur at the time of "Philadelphia" chromosome rearrangement, t(9;22), and they do not reflect genomic instability during disease progression. Emergence of such deletions later in the course of CML disease progression may question the validity of this conclusion. We describe here a patient who initially presented with profound leukocytosis and severe anemia. Flow cytometry results on patient's bone marrow biopsy suggested CML, and cytogenetics confirmed presence of t(9;22). Patient was started on Gleevac, 400 mg/day, with favorable initial response, but his platelet count started declining. A FISH evaluation using triple probe/3-color system (Vysis), confirmed presence of ABL/BCR rearrangement with normal probe signals for ASS, ABL and BCR genes on the derivative chromosome 9. In response to shifting platelet counts, Gleevac dose was periodically adjusted. A repeat FISH evaluation, three months later, again demonstrated ABL/BCR rearrangement, but the derivative chromosome 9 showed a deletion of the ABL gene. Abnormal cells (with BCR/ABL rearrangement) had increased from 65% to 85%. If the hypothesis that deletions of der(9) always occur at the time of Ph chromosome translocation event were true, two possible explanations are: (1) there were two clones to start, with the clone with del(9) not detected during the first FISH analysis, but it proliferated more rapidly, becoming the only detectable clone in three months, or (2) the clone with del(9) is a therapy related de novo occurrence with a proliferative advantage over the already existing clone without del(9). The alternate explanation is that the cell line with del(9) represents a clonal evolution from the parent clone without del(9), thus associated with a genomic instability during disease progression. Additional follow-up studies on CML patients, especially those refractory to Gleevac therapy, may help clarify this issue.

**Induction and expression of cyclin D3 in Korean Hepatocellular carcinoma.** *Y. Kang<sup>1</sup>, G. Kim<sup>2</sup>, K. Uhm<sup>1</sup>, S. Cho<sup>3</sup>, S. Park<sup>1</sup>.* 1) Institute of Human Genetics, Department of Anatomy, Brain Korea 21 BioMedical Sciences, Korea university College of Medicine, Seoul, Korea; 2) Perinatology Research Branch, National Institute of Child Health and Human Development, Department of Obstetrics and Gynecology, Wayne State University/Hutzel Hospital, MI, USA; 3) Department of Pathology, Hallym University, College of Medicine, Seoul, Korea.

Human cyclin D3 gene (CCND3) located on 6p21.1 is important in regulating the G1 checkpoint and involved in the regulation of the G1-S phase transition of the cell cycle. Overrepresentation of 6p21-p23 was frequently found in Korean hepatocellular carcinoma (HCC). We evaluated the intricate relationship between expression of cyclin D3 and the process of HCC development using immunohistochemistry and TUNEL assay on 43 paraffin embedded tissues. Cyclin D3 was mainly expressed in the nucleus, and in some cases, cytoplasmic staining was also detected. Cyclin D3 immunoreactivity was more frequently observed in the tumors with high histologic grade and the tumors with metastasis, and more frequently expressed in HCCs with cirrhotic background and gain of 6p21.1 when compared with those with non-neoplastic tissue. Apoptotic cells were more common in tumor with cirrhotic background, amplification of 6p21.1 and expression of cyclin D 3 when compared with HCCs with lower level of cyclin D3 expression. Also, some of the cyclin D3 positive cells and apoptotic cells were co-localized. From these results, it is suggested that over-expression of cyclin D3 may contribute to more rapid cell turn-over in the background of HCC, and balance between proliferation and apoptosis is a role in the progression of HCC with cirrhotic background.

**CHROMOSOME MICRODISSECTION AND FISH FOR THE IDENTIFICATION OF THE GENES**

**RESPONSIBLE FOR DRUG RESISTANCE.** *F. Mahjoubi*<sup>1</sup>, *G. Peters*<sup>2</sup>, *R. Hill*<sup>3</sup>. 1) Dr. F. Mahjoubi Dept. of Clinical Genetics, NRCGEB, Tehran, Tehran, Iran; 2) Dr. G. Peter Dept of Genetics, The New Children Hospital at Westmead, Sydney, Australia; 3) Dr. R. Hill Dept. of Molecular Sciences, CSIRO, Sydney, Australia.

The use of anticancer drugs in appropriate combinations has led to major improvements in the treatment of malignant disease. Unfortunately, in many cases such success is limited by the development of resistance to the chemotherapeutic drugs. Studies with model cell lines have revealed that simultaneous resistance to unrelated drugs, or multidrug resistance (MDR), can readily develop in mammalian cells. This raises the possibility that similar MDR tumor cells may also arise *in vivo* in human cancers, limiting a patient's response to chemotherapy. Our laboratory became interested in studying MDR cell sub-lines developed by treatment of the T-cell leukemia cell line CCRF-CEM with increasing levels of the anthracycline, epirubicin. The MDR phenotype is associated with an expanded chromosomal region (ECR). We have been interested in the nature of this extra DNA and the molecular events that give rise to amplification. As part of these studies, we have compared the content of the amplified unit in different derived E sub-lines. We microdissected this expanded region and PCR amplified the chromosomal DNA in order to produce sufficient DNA to determine its chromosomal origin and for use as a source of material to investigate candidate drug resistance genes. We demonstrated that the selection against epirubicin actually elevated the level of the multidrug resistance associated protein (mrp) gene. The probes prepared by chromosome microdissection should be useful for studying the organisation and function of DNA sequences within the ECR. We have also demonstrated a useful role for chromosome microdissection in this field of research.

**Leiomyosarcoma of the breast: a pathological and cytogenetic study of two cases.** *S. Li<sup>1</sup>, J. Lee<sup>1</sup>, M. Torbenson<sup>2</sup>, Q.Z. Liu<sup>3</sup>, J.J. Mulvihill<sup>1</sup>, B. Bane<sup>3</sup>, J. Wang<sup>3,4</sup>.* 1) Department of Pediatrics, OUHSC, Oklahoma City, OK; 2) Department of Pathology, Johns Hopkins University, Baltimore, MD; 3) Department of Pathology, OUHSC, Oklahoma City, OK; 4) Center for Advanced Diagnostics, AmeriPath Inc. Orlando, FL.

Leiomyosarcoma is an extremely rare form of primary breast tumor with up to 20 cases reported in the literature. In this report, we present the genetic findings of two cases of primary leiomyosarcoma of the breast. The patients were 44 years and 52 years of age and they presented with circumscribed masses ranging from 3.0 and 4.5 cm. Microscopically, the two tumors showed a diffuse proliferation of spindle cells arranged in short fascicles or bundles. There was moderate cytologic atypia and mitotic figures ranged from 6 to 12 per ten high power fields. The tumor cells were strongly immunoreactive for markers of smooth muscle differentiation including desmin, muscle specific actin, and smooth muscle actin. Comparative genomic hybridization (CGH) analysis was performed using DNA extracted from formalin-fixed paraffin-embedded tissue and showed losses of 10q (2/2 cases), 13q (2/2 cases), and 17q (1/2 cases), and gains of 1q (1/2 cases) and 17p (1/2 cases). Several known tumor related genes are located within regions including RB (13q14), BRCA2 (13q12), and PTEN (10q23). In addition, the patterns of chromosomal imbalances identified in leiomyosarcoma of the breast are similar to those reported in leiomyosarcoma of soft tissue and uterus and are different from those reported for leiomyoma, indicating that these alterations may be important for the development of malignant smooth muscle tumors regardless of site or organ of origin.

**Identifying the genes for paraganglioma, gastric stromal tumors (GST), and pulmonary chondroma (Carney triad/dyad): the SDHC gene and its locus.** *L.D. Matyakhina*<sup>1</sup>, *S. McWhinney*<sup>2</sup>, *T.A. Bei*<sup>1</sup>, *S. Stergiopoulos*<sup>1</sup>, *J.A. Carney*<sup>3</sup>, *C. Eng*<sup>2</sup>, *C.A. Stratakis*<sup>1</sup>. 1) DEB, NICHD, NIH, Bethesda, MD; 2) OSU, Columbus, OH; 3) Mayo Clinic, Rochester, MN.

\*The 3 first authors have contributed equally to this work.

Carney Triad (CT) describes the association of gastric stromal tumors (GST) with extra-adrenal paraganglioma (PGL), and pulmonary chondroma (PC); 79 non-familial cases of the triad have been reported; 67 female and 12 male. One-fifth of the patients had the three tumors; the remainder had two of the three, usually the gastric and pulmonary lesions. More recent evidence supports the possibility that the combination of paraganglioma and gastric stromal sarcoma may be familial, inherited as an autosomal dominant condition distinct from CT (Carney Dyad CD). Mitochondrial complex II, succinate dehydrogenase (SDH) consists of four subunits: SDHA, SDHB, SDHC and SDHD; of these the latter three are associated with familial and sporadic PGLs. Sporadic and familial GSTs are known to be associated with gain-of-function mutations of the oncogene *c-kit* (*CKIT*). In the present study, we investigated the possibility that patients with CT or CD and/or their tumors may harbor mutations of the SDH subunits and *CKIT* gene or genetic alterations of their genomic loci. A total of 26 tumors were investigated; 15 PGLs, 7GSTs and 4 PCs. A single tumor, a GST, was heterozygous for a point mutation (IVS5+1G>A) affecting the splicing of exon 5 of the *SDHC* gene. Polymorphisms in the 5' UTR of *SDHA* were also identified in several samples. Comparative genomic hybridization (CGH) demonstrated that the pattern of chromosomal changes was similar in benign PGLs and GSTs despite their different tissue origin and histology, consistent with a common genetic etiology underlying their formation. The most frequent and greatest contiguous change was deletion of the 1pcen13-q21 region, which harbors the *SDHC* gene. In aggregate, our results show that the genetic defects in the 1pcen-q21 region and/or the *SDHC* gene may cause this unique but potentially genetically heterogeneous disorder.

**Chromosome instability and copy number effect in tumors studied in microcell cell hybrid system.** *M. Kost-Alimova, E. Darai, A. Sandlund, L. Fedorova, Y. Yang, G. Klein, S. Imreh.* Dept MTC, Karolinska Inst, Stockholm, Sweden.

We demonstrated that microcell mediated chromosome 3 transfer into tumor renal cell carcinoma cells influences their in vitro and in vivo growth and directs the cells to a new round of chromosome alterations, which result in restoration of original tumorigenic phenotype. We show that the driving force behind this restoration includes chromosome copy number dependant instability and gene copy number dependant selection for loss providing growth advantage. Chromosome copy number dependant instability was manifested as an accumulation of imbalanced translocations between chromosomes, which were originally in more than two copies. Seven out of 9 trisomic chromosomes were found at least ones rearranged, while no one from 14 disomic chromosomes was altered. The translocation breakpoint lied preferentially in pericentromeric regions. Association of structural chromosome instability with hypersomy was evident also from analysis of available SKY data for a broad spectrum of human tumors. The pericentromeric imbalanced translocations were characteristic for hyperploid tumors, while tumors with near diploid karyotype favored balanced reciprocal translocations. Location of breakpoints within the pericentromeric regions may be at least partially associated with their late replication, because the other large latest replicating segments on the chromosome 3 were also involved in rearrangements. In this respect we demonstrated that hypersomy associated chromosome instability may be caused by asynchroneous replication of the additional loci and incomplete replication within the late replicating sequences. Chromosome breakage and then translocation was accompanied by segmental losses, which often involved a whole arm. Gene copy number dependant selection in our system was displayed as a clonal expansion in vivo and in vitro of cells loosing additional copies of 3p regions (in particular so called CER1 and FER at 3p14-21) caused as we show, by dose dependent proliferation advantage.

**Subtelomeric rearrangements in patients with pancytopenia.** *J. Lee, J.J. Mulvihill, S. Li.* Department of Pediatrics, OUHSC Oklahoma City, OK.

Many etiologies could cause pancytopenia, which is an abnormal condition of the reduction of red blood cells, white blood cells and platelets in the blood. A percentage of patients with pancytopenia have preleukemic disorders, and no specific chromosomal changes have been identified. To investigate whether these patients may have subtle chromosomal changes, especially the subtelomeric rearrangements, we have studied twelve patients with pancytopenia. Of these twelve patients, two cases had clonal chromosomal anomalies, and two cases had a normal karyotype but a low mitotic index. These four patients were excluded from further studies. The remaining eight patients were examined by fluorescence in situ hybridization, utilizing commercial probes corresponding to all of the subtelomeric regions of all 22 autosomes and the sex chromosomes, X and Y, except for the short arms of the acrocentric chromosomes 13, 14, 15, 21 and 22. None of the patients had a subtelomeric deletion, duplication or translocation. Our findings, although in a limited number of cases, may suggest low frequency of subtelomeric rearrangements in patients with pancytopenia.

**Cytogenetic characterization of uterine leiomyomata with t(10;17) involve the histone acetyltransferase *MORF*.** *S.D.P. Moore*<sup>1,2</sup>, *S.R. Herrick*<sup>1</sup>, *T. Ince*<sup>1,3</sup>, *P. Dal Cin*<sup>1,2</sup>, *M. Kleinman*<sup>1</sup>, *C.C. Morton*<sup>1,2</sup>, *B.J. Quade*<sup>1,2</sup>. 1) Brigham & Women's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Massachusetts Institute of Technology, Boston, MA.

Uterine leiomyoma (UL) are the most common tumor in women of reproductive age and the leading cause for hysterectomy in the United States. Cytogenetic aberrations that contribute to the pathobiology of this benign smooth muscle tumor primarily involve chromosomes 6, 7, 12, and 14. Rearrangements of 10q22 have also been reported and, interestingly, have also been observed in their presumed malignant counterpart, uterine leiomyosarcoma. We have mapped the chromosomal breakpoints in a UL with a t(10;17)(q22;q21) by fluorescence in situ hybridization (FISH). The chromosome 10 breakpoint localized to the third intron of the histone acetyltransferase *MORF* (monocytic leukemia zinc finger protein-related factor or *MOZ2*). *MORF* is a member of the histone acetyltransferase (HAT) MYST family, named for its founding members *MOZ*, *Ybf2/Sas3*, *Sas2*, and *Tip60*. Several cancers have been associated with rearrangement of HAT genes. For example, the closely related *MOZ* gene (monocytic leukemia zinc finger protein) has previously been found to be involved in acute myeloid leukemia. This is the first smooth muscle tumor to involve a disruption of a histone acetyltransferase. The t(10;17) disrupts *MORF* in the N-terminal portion of the protein between a conserved domain found in histone families 1 and 5 and PHD zinc finger domains. Interphase FISH analysis of three additional UL revealed disruptions in *MORF*. Two of these tumors had rearrangements between 10q22 and 17q21 or 17q22, one as a simple translocation and the other as a four-way translocation. The other tumor had two simple translocations involving 10q22 and 4q21 and 6p21 respectively. Of note, both tumors available for pathological review were of a particular histological subtype, namely the cellular variant. Cytogenetic mapping on chromosome 17 has identified candidate genes that may be associated with potential fusion products. The involvement of *MORF* in four UL suggests a role for this histone acetyl transferase in uterine mesenchymal neoplasia.

**A rare deletion of chromosome 20p in a patient with pancytopenia.** *M.J. Macera<sup>1</sup>, S.R.S. Gottesman<sup>2</sup>, V. Mizhritskaya<sup>1</sup>, R. Zeng<sup>1</sup>, A. Babu<sup>1</sup>.* 1) Dept Molec Medicine & Genetics, Wyckoff Heights Medical Ctr, Brooklyn, NY; 2) Dept of Pathology, SUNY Downstate Medical Center, Brooklyn, NY.

An 80 year old patient with a four year history of pancytopenia was evaluated for progressive worsening of his anemia. The bone marrow was found to be hypercellular with a left shift in myeloid maturation and mild dyserythropoiesis. Cytogenetic analysis on the bone marrow specimen revealed an abnormal chromosome 20, as the sole abnormality. Deletions of the 20q arm have been reported in numerous myeloid disorders, and is most frequently observed in refractory anemia and polycythemia vera, where its presence serves as a diagnostic tool used for disease classification. Deletions in the short arm of 20, however, are extremely rare and have not been reported as the sole abnormality. Two previous cases of acute lymphoblastic leukemia have been reported (Murphy et al, 1989; Walters et al, 1990) with del(20)(p11), both with additional abnormalities. Due to the rarity of a 20p deletion, whole chromosome 20 painting probe (wcp) and sub-telomeric probes specific for both the p (20ptel) and q (20qtel) terminals of chromosome 20 were applied to confirm and clarify the abnormality. FISH analysis revealed wcp (20) signal over the entire normal homologue and the entire del(20). No wcp20 signal was observed on any other chromosome. Signal for 20qtel was seen over the q terminal of the normal homologue and the del(20). However, signal for 20ptel was only observed on the normal homologue, no 20ptel signal was observed over the del(20) or anywhere else in the genome. Based on the banding profiles, the deletion break point is at or very close to the p11.1 and p11.2 interface, most probably within the proximal end of band p11.21, which was further confirmed by Comparative Genomic Hybridization (CGH). The final karyotype was 46,XY,del(20)(p11.2).ish del(20)(20ptel-,wcp20+,20qtel+). We are not aware of any previous case of a terminal deletion in the short (p) arm of chromosome 20 as the sole abnormality.

**Genetic imbalances of six urothelial carcinoma cell lines derived from a blackfoot endemic area in Taiwan.** C. Tzeng, C.P. Liou, M.Y. Chang. Department of Pathology, Chi-Mei Foundation Hospital, Tainan, Taiwan, Republic of China.

Unusually high incidences of urinary tract urothelial carcinoma (UC) have been reported from the endemic area for blackfoot disease in southern Taiwan, a unique peripheral vascular disease related to continuous arsenic exposure. However, chromosomal changes in such tumors, which may closely associate with tumor initiation and/or progression, have rarely been investigated. In this study, we applied comparative genomic hybridization to analyze six UC cell lines derived from patients with carcinoma of pT2 or higher stage in this endemic area. All lines displayed numerous chromosomal aberrations. Repeated gains and deletions (noted in two or more lines) were detected in 14 and 17 sites, respectively. The frequent gains were observed on 8q22-ter (6/6), 7q31-34 (3/6), 9p22-ter (3/6), 11q12-14 (3/6), and 20q11-13 (3/6). The common losses were noted at Xp11-22 (6/6), Xq24-ter (6/6), 4q13-23 (4/6), 4q28 (3/6), 5q11-14 (3/6), 10q23-25 (3/6), 11p13-15 (3/6), 11q14-ter (3/6), and 15q11-21 (3/6). Many of these aberrations have been previously described in the literature, especially gains of 8q and 20q and losses of 11p or/and q. However, our study showed some intriguing and uncommon regions, different from those found in past studies. These were the amplification of 7q31-34 and 9p22-ter and deletion of Xp11-22, Xq24-ter, and 15q11-21. In spite of the very complex pattern of genetic changes in these lines, most of these uncommon aberrations have to be implicated in UC, and further molecular genetic methods are necessary to establish whether the chromosomal regions contain candidate genes, which contributed to the initiation and progression of UC in the endemic area of Taiwan.

**A novel chromosomal translocation t(3;7)(q26;q21) in myeloid leukemia resulting in overexpression of *EVII*.** C. Storlazzi<sup>1,4</sup>, L. Anelli<sup>1,2</sup>, F. Albano<sup>3</sup>, A. Zagaria<sup>1,3</sup>, M. Ventura<sup>1</sup>, M. Rocchi<sup>1</sup>, I. Panagopoulos<sup>4</sup>, A. Pannunzio<sup>3</sup>, E. Ottaviani<sup>5</sup>, V. Liso<sup>3</sup>, G. Specchia<sup>2,3</sup>. 1) DAPEG, section of Genetics, University of Bari, Bari, Italy; 2) Hematology, University of Foggia, v.le Pinto, 71100 Foggia, Italy; 3) Department of Hematology, University of Bari, p.zza G.Cesare 11, 70124 Bari, Italy; 4) Department of Clinical Genetics, University Hospital, S 221 85 Lund, Sweden; 5) Institute of Hematology L. and A. Seragnoli, University of Bologna, via Massarenti 9, 40138 Bologna, Italy.

The *EVII* proto-oncogene encodes a nuclear zinc-finger protein that acts as a transcription repressor factor. In myeloid leukemia it is often activated by chromosomal rearrangements involving band 3q26, where the gene has been mapped. Here we report two leukemia cases [a Chronic Myeloid Leukemia Blast Crisis (CML BC) and an Acute Myeloid Leukemia (AML) M4] showing a t(3;7)(q26;q21) translocation in a balanced and unbalanced form, respectively. FISH analysis revealed that both patients showed a breakpoint on chromosome 3 inside the clone RP11-33A1 containing the *EVII* oncogene and, on chromosome 7, inside the clone RP11-322M5, partially containing the *CDK6* oncogene which is a D cyclin-dependent kinase gene, observed to be overexpressed and disrupted in many hematological malignancies. RT-PCR analysis showed overexpression of *EVII* in both cases but excluded the presence of any *CDK6/EVII* fusion transcript. *CDK6* expression was also detected. Together, these data indicate that *EVII* activation is likely due not to the generation of a novel fusion gene with *CDK6* but to a position effect dysregulating its transcriptional pattern.

**Genetic alterations in intrahepatic cholangiocarcinoma by degenerate oligonucleotide primed PCR-comparative genomic hybridization.** *K. Uhm<sup>1</sup>, Y. Kang<sup>1</sup>, S. Park<sup>2</sup>, Y. Park<sup>3</sup>, S. Park<sup>1</sup>*. 1) Institute of Human Genetics, Department of Anatomy, Brain Korea 21 Biomedical Sciences, Korea University College of Medicine, Seoul, Korea; 2) Department of Anatomy, Ehwa womans University College of Medicine, Seoul, Korea; 3) Department of Pathology, Yonsei University College of Medicine, Seoul, Korea.

Intrahepatic cholangiocarcinoma (ICC), a malignant neoplasm of the biliary epithelium, is usually fatal because of difficulty in early diagnosis and lack of availability of effective therapy. Furthermore, little is known about the genetics and biology of ICC. Chromosomal aberrations in 49 Korean ICC were investigated by degenerate oligonucleotide primed - PCR comparative genomic hybridization. The common sites of copy number increases were 20q (69%), 17q (66%), 19pter~q12 (57%), 11q11~q23 (54%), 22q (48%), 7q (39%), 16p (39%), 8q (36%), 15q22~q25 (36%), 18p (36%) and 6p21 (33%). DNA amplification was identified in 18 carcinomas (54%). The frequent sites of amplification were 7q11~q22, 8q24, 16p, 17q23~qter, 19, and 20q. Chromosomal loss was less common in our series of ICC and found in 1p34~pter, 3p, 4q, 5q, 6q, 13q, 18q, and X. The recurrent gains and losses of chromosomal regions identified in this study provide candidate regions that may contain oncogenes or tumor suppressor genes, respectively.

**The deletion of retinoblastoma region (13q13-q14) detected by CGH in a patient with malignant phyllodes tumor of the breast.** *J. Wang*<sup>1,4</sup>, *J. Lee*<sup>2</sup>, *M. Torbenson*<sup>3</sup>, *Q.Z. Liu*<sup>1</sup>, *J.J. Mulvihill*<sup>2</sup>, *S. Li*<sup>2</sup>. 1) Department of Pathology, OUHSC, Oklahoma City, OK; 2) Department of Pediatrics, OUHSC, Oklahoma City, OK; 3) Department of Pathology, John Hopkins University, Baltimore, MD; 4) Center for Advanced Diagnostics, AmeriPath Inc, Orlando, FL.

Phyllodes tumor of the breast, accounting for only 0.3% of all breast tumors, is a biphasic tumor composed of benign epithelial elements and a cellular, spindle-cell stroma. It is classified as benign, borderline or malignant categories based on the features of the stromal elements. Gain of 1q and the loss of 3p were considered the common chromosomal changes. However, no correlation between clinical presentation and cytogenetic findings was established. Here we report comparative genomic hybridization (CGH) findings in a case of unusual malignant phyllodes tumor. A 55-year-old patient presented with a circumscribed mass measuring up to 13.0 cm. The tumor showed biphasic stromal and epithelial growth. The stromal component was predominant, and spindle stromal cells were strongly immunoreactive for the markers of muscle differentiation, which is an unusual finding in phyllodes tumor. CGH analysis was performed using formalin-fixed, paraffin-embedded tissue. In addition to a gain of 1q, three additional imbalanced chromosome regions, a gain of 5p and the loss of 10p and 13q13-q14, were identified. Interestingly, the region of 13q14 harbors the RB1 tumor suppressor gene, and whether the deletion of RB1 gene in this case plays a role in development of malignant phyllodes tumor of breast warrants further investigation.

**Atypical morphologies of bone marrow due to a variant translocation (8;10;21) in a patient with acute myeloid leukemia.** *K.V. Scott<sup>1</sup>, W.F. Kern<sup>2</sup>, J.B. Parkhurst Cain<sup>1</sup>, J. Lee<sup>1</sup>, J.J. Mulvihill<sup>1</sup>, S. Li<sup>1</sup>.* 1) Department of Pediatrics, OUHSC, Oklahoma City, OK; 2) Department of Pathology, OUHSC, Oklahoma City, OK.

The t(8;21)(q22;q22) translocation is one of the most common nonrandom chromosomal changes in AML and is strongly associated with the M2 subtype of AML. The marrow cells show typical morphologic features that include prominent Auer rods, homogeneous salmon-colored granules and strongly myeloperoxidase-positive blasts. Rare variant translocations have been reported; all had similar AML M2 morphology. We report a patient who was initially diagnosed with a chronic myeloproliferative disorder, possibly chronic myelogenous leukemia (CML), based on laboratory and blood/marrow morphological findings. The patient did not respond well to treatment for CML. Chromosomal analysis revealed that the patient did not have t(9;22), but a complex variant translocation t(8;10;21). Treatment regime was switched to AML protocol and the patient responded well and she is now in remission. Reviewing published literature, it is suggested that the disruption of gene(s) on chromosome 10q24 may contribute atypical AML M2 morphologic changes in our case.

**CHROMOSOMAL ABNORMALITIES IN VENEZUELAN PEOPLE WITH MULTIPLE MYELOMA. A.E.**

*Rojas-Atencio<sup>1</sup>, M. Quintero<sup>2</sup>, J. Herrera-Cepeda<sup>2</sup>, K. Urdaneta<sup>1</sup>, M. Soto<sup>1</sup>, P. Garcez<sup>1</sup>, F. Alvarez-Nava<sup>1</sup>.* 1) Genetica, Universidad del Zulia, Maracaibo, Zulia, Venezuela; 2) Servicio Hematologia, Universidad del Zulia.

The cytogenetic study is an important prognostic factor in Multiple Myeloma (MM) study, the chromosomal analysis demonstrated to be essential for the genetic advise in relation to the diagnosis, prognostic and suggesting precociously, the most appropriate treatment for the most number of hematological malignancies. The objective of this work was to identify the chromosomal abnormalities in samples of bone marrow (BM) coming from patients with diagnosis of MM in our region. The chromosomal studies were carried out in cultures of BM, following the technique described by J. Yunis in 1981, without exception it was carried out previous to any treatment with cytostatics. Twenty two samples of BM were received for chromosomal study in the Unit of Medical Genetics of the University of the Zulia (UGM -LUZ), 19/22 samples (86,36%) appropriate material was obtained by cytogenetic analysis, 6/19 (31,57%) normal karyotype, 13/19 (68.42%) they were numeric and structural chromosomal abnormalities. 100% of the chromosomal anomalies observed were compound for numerics 8 (42,10%), three case with hyperdiploidy involving chromosomes 3, 5, 7, 15, 17, 18, 19, four case with hypodiploidy involving the chromosomes 8, 16, 17, 18, X and Y. Triploidy in one case. The structural abnormalities in 4 cases (33.33%) as the deletion 5p11, 11p14, 14q32, 17p11 and 1 (8.33%) presented structural and numeric anomalies. Our study shows that their majority the patients with multiple myeloma present some type of chromosomal abnormalities, and variability in the frequency of this alterations.

**Clonal evolution in a patient with secondary myelodysplastic syndrome.** A. Podroma<sup>1</sup>, J. Sanmugarajah<sup>1</sup>, I. Yudelman<sup>1</sup>, M.J. Macera<sup>2</sup>, R. Zeng<sup>2</sup>, A. Babu<sup>2</sup>. 1) Department of Medicine, Lenox Hill Hospital, New York, NY; 2) Division of Molecular Medicine and Genetics, Department of Medicine, Wyckoff Heights Medical Center, Brooklyn, NY.

A 55 year old female patient, 5 years ago, had a modified radical mastectomy for stage IIb breast cancer and underwent chemotherapy with Cytosan, Adriamycin, Fluorouracil (cyclex4) followed by autologous bone marrow transplant. The patient has been experiencing anemia for 8 months followed by 2 months of leucopenia and thrombocytopenia. Her bone marrow biopsy was hypercellular with 11% blasts. Chromosome analysis on bone marrow revealed two abnormal clones: a stemline with a deletion of chromosome 5q, 46,XX,del(5)(q14q34), in 13% and a sideline (the mainline), with several additional abnormalities, 54,idem,+1,del(6)(p21.2),+del(6)(p21.2),+8,+10,+11,+14,+15,+22, in 87% of the metaphase cells. A subsequent analysis on a peripheral blood specimen, after one week, showed only the sideline and a single normal cell. No stemline was detected in the metaphase cells of the unstimulated blood specimen. Approximately 80% of therapy related Myelodysplastic syndromes (t-MDS) show chromosomal abnormalities. These abnormalities are mostly in the form of deletions or additions and seldom are in the form of chromosome rearrangements. The present case is an additional example towards that observation. While prognosis associated with a primary event such as 5q- as a sole abnormality is good, additional abnormalities greater than 3 is considered as a basis for poor prognosis (Greenburg et al, 1997). The patient is scheduled to begin treatment with 5 Azacitidine. Additional follow up data is essential to determine the aberrations contributing towards clonal evolution, their implications on disease progression and evaluating therapeutic efficacy.

**Detection of ins(4;3)(q21;q12q27) by FISH in a case of MDS/AML with a del(5)(q13q32) and monosomy 7. D.**

*Wei<sup>1</sup>, K.H. Ramesh<sup>1</sup>, V.R. Pulijaal<sup>1</sup>, M. Zohouri<sup>1</sup>, D. Walsh<sup>1</sup>, A. Deshikar<sup>1</sup>, L.V. Vasovic<sup>1</sup>, H. Ratech<sup>1</sup>, D.S. Xu<sup>1</sup>, E.W. Friedman<sup>2</sup>, L.A. Cannizzaro<sup>1</sup>.* 1) Pathology, Montefiore Medical Center, Bronx, NY. 10461; 2) Medicine, Div. Hematology-Oncology, Montefiore Medical Center, Bronx, NY. 10467.

An 85 year-old female with a history of thrombocytosis was diagnosed with myeloproliferative disorder (MPD) in 1996. She was treated with P in 1998 and Hydrea since then. In Oct. 2002, she had worsening anemia and a leucocytosis. Flow cytometry detected 8% CD34+ myeloblasts with decreased side-scatter of mature myeloid cells. A bone marrow (BM) biopsy showed hypercellular marrow (80-100%) with absolute granulocytic and megakaryocytic hyperplasia and diffuse increase in reticulin fibers (3+/4+). In April 2003 her BM was hypercellular (80%) with hiatus in myeloid maturation and diffuse fibrosis. Flow cytometry showed an increase 12-18% CD34+, CD11b+, CD11c+, CD13+, CD33+, CD38+ and HLA-DR+ immature myeloid precursors. Peripheral blood (PB) showed an elevated WBC (94 k/ul) with blasts (Hb 5.4 g/dl, MCV 109fL, PLT 249K/CU, LDH 1848 U/L). The differential diagnosis now included refractory anemia with excess blasts (RAEB) versus acute myeloid leukemia (AML) with prior MDS. Cytogenetic and FISH analyses of BM (10/02) revealed a 46,XX,ins(4;3)(q21;q12q27),del(5)(q13q32),-7,add(18)(p11.2),+mar[20]. ish ins(4;3)(q21;q12q27)(WCP3+) karyotype. Anomalies in all the cells included a partial insertion of 3q into 4q (confirmed by FISH), an interstitial deletion of 5q, loss of 7, additional unknown chromosomal material in 18p, and an unidentifiable marker chromosome. Deletions of 5q and loss of 7 have been reported in MDS, AML, RAEB, and in MPD. However in this patient there were secondary numerical and structural chromosomal changes that continued to evolve. In April 2003, there was a terminal deletion of Xq and two copies of the add(18) anomaly, while the other abnormalities remained unchanged. The presence of deletion 5q and loss of 7, in association with other multiple chromosomal changes, are suggestive of secondary MDS/AML in this patient.

**A t(1;22)(p13;q13) in an infant with acute megakaryoblastic leukemia (M7).** *R. Sundaram<sup>1</sup>, D. Rakhman<sup>1</sup>, R. Alexis<sup>1</sup>, A. Babu<sup>2</sup>, R. Zeng<sup>2</sup>, S. Kleyman<sup>2</sup>, M.J. Macera<sup>2</sup>.* 1) Long Island College Hospital, Brooklyn, NY; 2) Division of Molecular Medicine and Genetics, Department of Medicine, Wyckoff Heights Medical Center, Brooklyn, NY.

Approximately 20% of infants afflicted with AML have Acute Megakaryoblastic Leukemia (FAB classification M7). One subgroup of M7, based upon cytogenetic criteria are those patients that have the t(1;22)(p13;q13) karyotype. This group consists almost exclusively of infants, as the translocation is seldom described in children over 1 year of age. They frequently exhibit hepatosplenomegaly and show a constant presence of both blasts and megakaryocytes. Their prognosis is uniformly poor. To date there are only approximately 40 cases reported in the literature. A male child weighing 3284 gm was the product of a 39 week gestation, delivered by repeat cesarean section to a 39 year old mother. The pregnancy was complicated with a three day hospitalization for spotting and possible urinary tract infection, for which the mother received intravenous antibiotics for three days. At birth, the baby had a platelet count of 60-70,000. Three days later, his platelets had dropped to 22,000. Physical examination revealed hepatosplenomegaly and his peripheral smear revealed occasional blasts. A bone marrow revealed 12% blasts with absence of megakaryocytes. As he had guiac positive stools, he received a platelet transfusion and intravenous gamma globulin for five days. Two weeks later, a repeat marrow showed 24% blasts. Cytogenetic analysis on unstimulated peripheral blood revealed a 46,XY,t(1;22)(p13;q13)[7]/46,XY[13] mosaic. Only normal 46,XY cells were found in PHA stimulated blood, thus documenting the acquired nature of the 46,XY,t(1;22)(p13;q13) clone and establishing the M7 diagnosis. Treatment was initiated using standard chemotherapy but the patient passed away within six weeks of diagnosis. Although megakaryocytes were not documented in the marrow, this child's disease presentation: the presence of blasts, hepatosplenomegaly and having a poor prognosis, strengthens the M7 category of Acute Megakaryoblastic Leukemia in infants with the t(1;22)(p13;q13) translocation.

**A case of myelodysplasia syndrome with an unusual 46,XY,t(20;22)(q13;q11.2) karyotype.** *M. Sinha*<sup>1</sup>, *M.J. Macera*<sup>2</sup>, *V. Mizhiritskaya*<sup>2</sup>, *R. Zeng*<sup>2</sup>, *A. Babu*<sup>2</sup>. 1) Department of Oncology, Forest Hills Medical Center, Forest Hills, NY; 2) Division of Molecular Medicine and Genetics, Department of Medicine, Wyckoff Heights Medical Center, Brooklyn, NY.

A 66 year old male with a history of low platelets was referred for thrombocytopenia 5 years ago. His platelets were 44k and a presumptive diagnosis of idiopathic thrombocytopenic purpura (ITP) was made. He was started on Prednisone with an excellent initial response, followed by a fall that required gamma globulins. He was continued on steroids in tapering doses and gamma globulins for 2 years, at which time, with elevated WBC, his symptoms were suggestive of myelodysplasia (MDS) or unusual chronic myelogenous leukemia (CML). He was continued on steroids and IVIG for 4 years. Cytogenetic analysis of a recent bone marrow revealed a translocation involving chromosomes 20 and 22. FISH was performed using probes for BCR/ABL, wcp 20 and the subtelomere specific regions of the short and long arms of chromosome 20 (20ptel, 20qtel). Probe for ABL was seen only over both 9s, while BCR signal was seen over the normal 22 and the der(20), establishing one breakpoint at 22q11.2. Signal for wcp 20 was found over the entire normal homologue, half of the der (20) and a small band located at the distal end of the der (22). Signal for the 20ptel probe was seen over the p arm of the normal 20 and over the der (20), while signal for the 20qtel was seen over the normal 20 and the der 22. Based on these findings, the karyotype was designated as 46,XY,t(20;22)(q13;q11.2). ish 9q34(ABLx2), der(20)(BCR+,wcp20+,20ptel+,20qtel-), der(22)(BCR-,wcp20+,20qtel+). The t(20;22)(q13;q11) translocation is extremely rare and has only been reported twice in two cases classified as CML with aberrant translocations. The BCR/ABL fusion gene was documented in one case (Ito et al 2002), establishing the involvement of ABL, located at 9q34, in the translocation. No information is available regarding BCR/ABL status in the second reported case (Becher et al 1987). Since no involvement of the ABL gene was found, this case presents an unusual translocation, t(20;22)(q13;q11.2), with unique breakpoints, not involving ABL or chromosome 9.

Program Nr: 323 from 2003 ASHG Annual Meeting

**Jumping translocation involving chromosome 21 in clear cell renal carcinoma.** *C. Sreekantaiah*. Dir, Cytogenetics Lab, Lab Diagnostics, LLC, Norwalk, CT.

Jumping translocations are rare cytogenetic aberrations that involve one donor chromosome and multiple recipient chromosomes. A nonclonal jumping translocation involving chromosome 21 was noted as the only structural rearrangement in a renal cell carcinoma, clear cell type. The translocation partners were the telomeric regions of 12p, 13p, 20q, and 21q. The karyotype of the tumor was 68-76<4n>,XX,-X,-X,-1,-1,-2,-2,-6,-6,-8,-8,-10,-10,-17,-17,-21,-21[cp8]/46,XX[12].

**Multiple Cytogenetic Abnormalities including a t(11:17)(q23;q21) in a Patient With de novo CD34 Negative Acute Myeloid Leukemia (AML).** *M. Zohouri<sup>1</sup>, L.A. Cannizzaro<sup>1</sup>, D. Wei<sup>1</sup>, V.R. Pulijaal<sup>1</sup>, D. Walsh<sup>1</sup>, T. Zhou<sup>1</sup>, L.V. Vasovic<sup>1</sup>, H. Ratech<sup>1</sup>, D.S. Xu<sup>1</sup>, B. Pimperdi<sup>2</sup>, S. Aggarwal<sup>2</sup>, L.I. Cytryn<sup>3</sup>, K.H. Ramesh<sup>1</sup>.* 1) Pathology, Montefiore Medical Center, Bronx, NY; 2) Oncology, Montefiore Medical Center, Bronx, NY. 10467; 3) Medicine, Div.Hematology-Oncology, Montefiore Medical Center, NY. 10467.

A 26 year old female reported dizziness for a period of one week in 2/2003. Peripheral blood analysis showed pancytopenia (Hb 7.2 g/dl, WBC 0.9, PLT 136K/CU) with rare blasts. Bone marrow (BM) was hypercellular marrow (100%), almost completely replaced with blasts and no normal hematopoiesis. Flow cytometry revealed CD34-, CD117+, CD33+, CD13- CD11b+, CD11c+, HLA-DR+, CD4+, CD7 (weak partial)+, TdT (weak to negative), consistent with an aberrant immature myeloid phenotype. Cytochemical enzyme studies showed that the blasts were positive for alpha-naphthyl-butyrate esterase activity. The immunohistochemical stains revealed that 50% of the blasts were myeloperoxidase positive and 75-100% were CD68 positive. Based on these findings, a diagnosis of AML with monocytic differentiation was made. Cytogenetic analysis of BM revealed a 45,XX,del(1)(p13),t(11;17)(q23;q21),-15[12]/73,idemx2,+1,+16,+20,+22[7]/46,XX [1] karyotype. In the first clone, anomalies included a terminal deletion of 1p, a translocation involving chromosome bands 11q23 and 17q21 and monosomy 15 (12/20 cells). The second clone had anomalies similar to the first, but the count was 73, with all chromosomes in 3 copies except 1, 16, 20 and 22, (4 copies)(7/20 cells). FISH with the MLL, PML/RARA and the 11p and q subtelomere probes was performed to characterize the cytogenetic anomalies. FISH proved that the break occurred at the MLL gene locus. PML/RARA confirmed loss of one 15, and translocation of RARA to 11q23. She received induction therapy with Arac and Idarubicin and achieved complete remission. After one cycle of high dose Arac she underwent allogenic BMT from a matched unrelated donor. Structural abnormalities involving the MLL gene are seen in 5-10% of acquired chromosomal rearrangements in hematological malignancies and are predictive of a poor prognosis.

**Acquired homozygosity for t(9;22) during clonal evolution in CML.** *L. Wisniewski<sup>1</sup>, P. Papenhausen<sup>1</sup>, J. Tepperberg<sup>1</sup>, P. Mowrey<sup>1</sup>, P. Singh-Kahlon<sup>1</sup>, I. Gadi<sup>1</sup>, L. Moscinski<sup>2</sup>.* 1) Cytogenetics, LabCorp of America, RTP, NC; 2) H.Lee Moffitt Cancer Center, Tampa, FL.

We wish to report an unusual case of acquired homozygosity for t(9;22) associated with clonal evolution of CML in a 57-year-old male. Previous FISH studies with BCR/ABL fusion probes performed at diagnosis and at six-month follow-up established the diagnosis of CML. Fusion signals were noted in approximately 60% of the cells examined, and conventional cytogenetic studies revealed 46,XY,t(9;22)/46,XY mosaicism on both occasions. There was no evidence of abnormalities associated with clonal evolution in either evaluation. Cytogenetic studies performed one year after diagnosis revealed a previously undetected side line with the karyotype 46,X,+X,-Y,+8,+8,-9,t(9;22)(q34;q11.2)x2,-22 in 10 of 20 metaphases analyzed. Both cell lines observed in the previous studies were also represented. Chromosome heteromorphisms in the three cell lines indicated that the side line was derived from the t(9;22) stem line, and that homozygosity for the translocation was most likely achieved by mitotic nondisjunction leading to the loss of both normal 9 and 22 homologs and duplication of both translocation derivatives. The same mechanism appeared to have also resulted in acquired homozygosity for the X chromosome. It was not clear whether the tetrasomy 8 also noted arose by the same or a different mechanism. The patient, who had developed a myeloid blast crisis and was completely refractory to induction therapy, expired three weeks after the final cytogenetic study. Although previous reports have documented that one or both derivatives of the standard t(9;22) seen in CML may be duplicated at the time of diagnosis or later, this is the first observation to our knowledge of duplication of the translocation with simultaneous loss of both normal homologs during clonal evolution. The selective advantage of the unusual sideline however, can be attributed to the presence of classic CML secondary abnormalities (duplicated Ph', +8, +8) rather than the observed homozygosity.

**A novel subtelomeric translocation t(5;9) and the deletion of RB1 gene in a patient with acute myeloid leukemia (AML-M0).** *R.K. Taylor<sup>1</sup>, J. Lee<sup>1</sup>, W.F. Kern<sup>2</sup>, J.J. Mulvihill<sup>1</sup>, S. Li<sup>1</sup>.* 1) Department of Pediatrics, OUHSC, Oklahoma City, OK; 2) Department of Pathology, OUHSC, Oklahoma City, OK.

No chromosomal rearrangements have been identified to be specifically associated with minimally differentiated acute myeloid leukemia (AML-M0). Several research groups studied the cytogenetic features of AML-M0 and found that approximately 81% of patients had chromosomal rearrangements; primarily -5/5q- and/or 7/7q- deletion or translocations involving 12p. A patient, who was diagnosed with AML eighteen months ago, was referred for cytogenetic evaluation for possible AML relapse. The routine chromosome analysis was performed on bone marrow specimen and showed an apparently normal karyotype. Incidentally, this patient was enrolled in the study of subtelomeric changes in patients with pancytopenia. Fluorescence in situ hybridization (FISH) was performed utilizing commercial probes corresponding to the subtelomeric regions of all 22 autosomes and the sex chromosomes, X and Y, except for the short arms of the acrocentric chromosomes 13, 14, 15, 21 and 22. Each of the cells analyzed had a t(5;9) (q35.3;q34.3), plus a deletion of RB1 gene. To rule out the possibility that these chromosomal changes were related to the relapse of AML in this case, we repeated the same FISH test on the specimen at initial diagnosis before any treatment. The same abnormalities were found. To our knowledge, this is the first case reported with subtelomeric t(5;9) (q35.3;q34.3) and the deletion of RB1 gene in a patient with AML-M0. Whether the translocation (5;9) combined with the deletion of RB1 gene plays an important role in the development of AML-M0 warrants further investigation.

**Relationship of chromosome 21 rearrangements with other numerical chromosome changes in patients with acute lymphoblastic leukemia (ALL).** *L. Zhang<sup>1,2</sup>, J.J. Mulvihill<sup>1</sup>, S. Li<sup>1</sup>.* 1) Dept Pediatrics, OUHSC, Oklahoma City, OK; 2) Dept Hematology, The First Affiliated Hospital of China Medical University, Shenyang, P.R.China.

Numerical or structural changes of chromosome 21 have been recognized to be very common in patients with ALL and have values of predicting the outcome of treatment. Some of these structural rearrangements are very difficult, even impossible to be diagnosed by routine chromosome analysis, such as t(12;21), but easily done by fluorescence in situ hybridization (FISH) utilizing a TEL/AML1 gene fusion probe. Other chromosomal changes, such as trisomies 4, 10, 17, an MLL gene rearrangement, or t(9;22), also have implication of predicting the outcome of therapy. We have studied fifty-one patients with ALL utilizing multiple locus specific DNA probes TEL/AML1, MLL, BCR/ABL and centromere probes of chromosomes 4, 10 and 17; forty-five were pediatric patients and the remaining six were adults. The male to female ratio is 2:1. All of the probes used were purchased from a commercial source (Vysis, Downers Grove, Illinois, USA). Twenty-five out of 51 patients (49%) had either structural or numerical changes of chromosome 21. The patients who had structural changes, such as t(12;21) or a ring chromosome 21, did not have numerical changes of chromosome 21, 4, 10 or 17. We believe these are two separate mechanisms that lead to the development or progression of ALL.

**Cytogenetic characterization and *p53* tumor suppressor status of SW480 human adenocarcinoma cells. P.J.**

*Rochette*<sup>1</sup>, *J. Lavoie*<sup>1</sup>, *W. Dridi*<sup>1</sup>, *N. Bastien*<sup>1</sup>, *M. Bronsard*<sup>1</sup>, *E.A. Drobetsky*<sup>2</sup>, *R. Drouin*<sup>1</sup>. 1) Unité de Recherche en Génétique Humaine et Moléculaire, Research Center, Hospital Saint-François d'Assise, CHUQ, Laval University, Quebec, QC, Canada; 2) Guy-Bernier Research Center, Maisonneuve-Rosemont Hospital, University of Montreal, Montreal, QC, Canada.

It has been clearly demonstrated that cells lacking functional *p53* are defective in nucleotide excision repair of UV-induced DNA damage. Nonetheless, we have previously found that the model *p53*-deficient human tumor strain SW480 unexpectedly exhibits normal rates of DNA repair. Towards understanding the basis for this exceptional phenotype, we aimed to characterize the precise *p53* status of SW480. Although this strain is known to bear mutations in codons 273 and 309 of *p53*, it remains unclear how many *p53* alleles it carries and whether any of these alleles are wild type. Using GTG banding and M-FISH (multi-color fluorescence in situ hybridization), we found that SW480 cells are hyperdiploid and polyclonal, but are cytogenetically stable. We have characterized many structural chromosome anomalies. Furthermore, they have three copies of chromosome 17 (i.e., 17p13.1, where *p53* is localized). The integrity of the short arm of chromosome 17 in SW480 was confirmed using FISH with probes specific for Smith-Magenis (17p11.2) and Miller-Dieker (17p13.3) syndromes. Using DNA sequencing and restriction analysis, it was revealed that all three *p53* alleles in SW480 carry point mutations in codons 273 and 309. By in vivo footprinting using ligation-mediated PCR, we are currently analyzing the ability of this mutated *p53* protein to bind the *p53* consensus sequence located at target genes such as XPC, p21, p48, GADD45.

**FANCD2 associates with BLM at telomeres in ALT-immortalized human cells.** *H. Root*<sup>1, 2</sup>, *M.S. Meyn*<sup>1,2,3</sup>. 1) Genetics and Genomic Biology, Hospital for Sick Children, Toronto, ON; 2) Molecular & Medical Genetics, University of Toronto, Toronto, ON; 3) Paediatrics, University of Toronto, Toronto, ON.

Fanconi anemia patients have short telomeres, which correlate with genomic instability and bone marrow failure. ~10% of tumors lack telomerase and maintain their telomeres by ALT pathways, which may involve genetic recombination. We recently found that BLM, the Bloom syndrome DNA helicase, is involved in ALT. We now report data supporting a similar role for the Fanconi anemia protein FANCD2.

FANCD2 normally forms nuclear foci. In 1/3 of asynchronously growing ALT human fibroblasts, a majority of these foci colocalized with foci of BLM and with foci of TRF1, a component of telomeres. Association of FANCD2 with telomeric foci was ALT-specific, as FANCD2 and TRF1 rarely co-localized in telomerase-positive cells. FANCD2 frequently associates with BRCA1. However, in ~50% of cells showing FANCD2 colocalization with TRF1, FANCD2 foci did not colocalize with BRCA1. In addition, BRCA1 foci rarely associated with telomeric foci except when colocalized with FANCD2. This suggests that FANCD2 is not carried to telomeric foci by BRCA1, but BRCA1 may be at telomeres as part of a complex with FANCD2.

In synchronized cultures, ~90% of S-phase cells showed a majority of FANCD2 foci colocalizing with BLM and TRF1, indicating that FANCD2 and BLM preferentially associate with telomeric foci during DNA replication. The association of FANCD2 with TRF1 in ALT cells is not solely due to its localization to PML bodies, as FANCD2 colocalizes with PML in only ~50% of ALT cells in early S phase. We now are using IP and FRET techniques to test for *in vivo* interactions between FANCD2, BLM and TRF2.

We transfected fibroblasts with shRNA vectors designed to suppress FANCD2 expression and found that 8/30 telomerase-positive transformants, but 0/82 ALT transformants lacked or had markedly reduced FANCD2 expression by Western blotting, suggesting that FANCD2 may be required for proper ALT function. Our results support a model in which FANCD2 acts with BLM to facilitate recombination-driven replication of telomeres in ALT cells.

**Genomic instability in TP53-mutated breast cancer cells treated with etoposide and doxorubicin.** *M.L. De Vos, B.L. Weber.* Hematology/Oncology, University of Pennsylvania, Abramson Family Cancer Research Institute, Philadelphia, PA.

Mutations in the TP53 gene are among the most common genetic changes in cancer cells, with 20-40% of breast cancers having somatic TP53 mutations. It is hypothesized that when TP53 mutations are present in cancer cells, treatment with chemotherapeutic agents that induce double strand breaks (DSBs), such as doxorubicin and etoposide, results in greater genomic instability than in the presence of wildtype-TP53 (WT-TP53). Therefore, we are investigating the gene copy number alterations following etoposide and doxorubicin treatment of breast cancer cells with either WT- or mutated-TP53 (mut-TP53). Evaluation of the cytotoxicity of doxorubicin in MCF-7 (WT-TP53), MDA-MB-231 (mut-TP53), and T-47D (mut-TP53) breast cancer cells demonstrated that cells with WT-TP53 are more sensitive to doxorubicin than cancer cells with mutated TP53. Specifically, the IC<sub>50</sub> for cytotoxicity of doxorubicin at 24 hours was 2.0-0.8 M in MCF-7 cells, 41-7.4 M in MDA-MB-231 cells, and 97.5-31.8 M in T-47D cells. Similarly, 0.2-0.8 M doxorubicin treatment for 24 hours resulted in a larger number of DSBs in the TP53-mutated cells, as measured by  $\gamma$ -phosphorylation of histone H2AX. Finally, genomic stability in WT- and mut-TP53 cells treated with DSB-inducing chemotherapeutics will be determined using array-based comparative genomic hybridization (CGH). By determining the genomic changes in WT- and mut-TP53 cells following induction of DSBs, we will evaluate whether the DSB induction by chemotherapeutics results in random or specific gene copy number changes and determine whether these copy number changes are associated with changes in gene expression. These data may provide insight into the relative chemotherapeutic resistance of human breast cancers with TP53 mutations and allow identification of genetic factors involved in the development of drug resistance in these patients.

**A case of myelodysplastic syndrome (MDS) with a constitutional mosaicism for trisomy 21 in the skin sample of a child.** *J. Hu<sup>1,2</sup>, S. Shekhter-Levin<sup>1</sup>, P. Shaw<sup>2</sup>, C. Bay<sup>2</sup>, S. Kochmar<sup>1</sup>, U. Surti<sup>1,2</sup>.* 1) Pittsburgh Cytogenetics Lab, UPMC Magee-Womens Hosp, Pittsburgh, PA; 2) University of Pittsburgh School of Medicine, Pittsburgh, PA.

A 3 year old female presented with anemia, thrombocytopenia and blasts in the peripheral blood (PB). A bone marrow aspirate revealed MDS and was analyzed for cytogenetic abnormalities. Twenty two G-banded metaphases were analyzed from 24-hour harvests of unstimulated bone marrow. A mosaic abnormal female karyotype 46,XX,t(1;19)(q42;p13.1)[12]/47,idem,+21[3]/47,idem,+21,-7,+mar[7] was obtained. The major clone had a balanced translocation between the long arm of chromosome 1 and the proximal short arm of chromosome 19. The second clone had trisomy 21 in addition to the 1;19 translocation. The third clone revealed a loss of one chromosome 7, replaced with a marker chromosome of unknown origin, in addition to the 1;19 translocation and trisomy 21. Complete or segmental monosomy 7 and trisomy 21 has been reported in childhood MDS and is associated with unfavorable prognosis. Because the t(1;19)(q23;p13.3) was seen in every cell analyzed, we performed cytogenetic analysis on the patient's PB to rule out a constitutional abnormality. A karyotype 46,XX,t(1;19)(q42;p13.1) was found in all 20 cells analyzed, confirming the constitutional origin of this translocation. Cytogenetic analysis was also performed on skin fibroblasts. In addition to all cells having the t(1;19)(q23;p13.3), 5 cells out of 50 from 2 separate cultures contained an extra chromosome 21. The presence of 2 cell lines in multiple cultures indicates that the patient is a true low level mosaic for trisomy 21. These findings indicate the trisomy 21 found in her bone marrow is a constitutional abnormality and the leukemic clone arose from this trisomic hematopoietic precursor. The child appeared phenotypically and developmentally normal on clinical evaluation except for slightly up slanted palpebral fissures and small increased space between her 1st and 2nd toes. Growth and development to date are normal. Molecular analysis will be performed to determine meiotic or postzygotic origin of the trisomic cells.

**Detection of urothelial carcinoma: Correlation between cytology and fluorescence in situ hybridization.** *D.J. Wolff<sup>1</sup>, T. Keane<sup>2</sup>, C. Felicissimo<sup>3</sup>*. 1) Dept. Pathology/Lab Med, Medical Univ South Carolina, Charleston, SC; 2) Dept. Urology, Medical Univ South Carolina, Charleston, SC; 3) Diagnostic Genetic Sciences Program, University of Connecticut, Storrs, CT.

Urothelial carcinoma (UC) is a common cancer in the United States with >50,000 new cases reported annually. UC has a high recurrence rate and urine cytology is the standard method employed for recurrence monitoring. However cytology has a low sensitivity, particularly for low-grade disease. The FDA-approved Vysis UroVysion Bladder Cancer Recurrence kit (UroV) uses DNA probes to detect chromosomal abnormalities frequently associated with UC. We hypothesized that UroV would have a higher detection sensitivity for UC in our unselected clinic population. 100 samples were processed by FISH and urine cytology. Results were concordant in 88% of cases and discrepant for 12%; 6 cases had insufficient cells for FISH analysis. 9 cases had negative cytologic findings, but were positive by FISH. Two cases were positive by cytology and negative by FISH. Cases with conflicting findings were further clarified with surgical pathology reports and/or physicians' diagnoses. UroV results were confirmed for all but three of the discrepant cases, one questionable false positive and two cases undergoing further studies. For the 19 cases with documented neoplasia, 18 had an abnormal FISH result (overall sensitivity = 95%). This was significantly higher than the 42% sensitivity obtained with cytology. While UroV is FDA-approved only for detection of recurrent UC, 58% of our cases had FISH testing as a screen for primary UC or primary or recurrent renal or ureteral carcinoma. Diagnosis for these cases was based on traditional cytology and surgical pathology, however results were consistent with those obtained with UroV and were thus included in the overall sensitivity. UroV testing allowed for early detection of UC, consistent with the fact that chromosomal abnormalities typically occur before the formation of detectable lesions or tumors. We conclude that UroV is a sensitive detection method for recurrent urothelial carcinoma and may be useful in the early detection of UC, as well as upper urinary tract carcinomas.

**Application of Fluorescent In Situ Hybridization (FISH) to detect *BCR/ABL* fusion in patients with chronic myelogenous leukemia.** *M. Soto Quintana, A. Rojas-Atencio, F. Álvarez-Nava, K. Urdaneta.* Unidad Genética Médica, Universidad del Zulia, Maracaibo, Venezuela.

Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disorder of the hematopoietic stem cell, clinically characterized by an overproduction of granulocytic cell. It is cytogenetically characterized by the presence of the Philadelphia (Ph) chromosome that arises from t(9;22)(q34;q11) or variant of this translocation. The translocation fuses the *ABL* and *BCR* genes by inserting the *ABL* proto-oncogen located at 9q34.1 to the *BCR* region at 22q11.2. This chimerical gene codes for an abnormal protein which has tyrosine kinase activity and significant leukemogenic potential. The objective of this study is to demonstrate the useful application of FISH in the identification of the *BCR-ABL* molecular complex in patients with CML. Bone marrow samples from 30 patients with CML were analyzed. To determine the *BCR-ABL* rearrangement by FISH we used the probe *LSI BCR/ABL* and hybridization protocol that were supplied by *VYSIS*. The standard cytogenetic techniques showed that 16 patients presented the t(9;22)(q34;q11), 6 patients showed a variant translocation of this anomaly and 8 patients normal karyotypes. FISH detected *BCR-ABL* fusion in all patients. Our study demonstrates the significance of this assay in the identification *BCR-ABL* fusion in patients with CML. Thus, FISH is a crucial to the accurate diagnosis and correct medical management.

**Incidence of variant FISH results in pediatric ALL.** *M. Thangavelu*<sup>1</sup>, *PA. Mowery-Rushton*<sup>2</sup>, *JL. Murata-Collins*<sup>2</sup>, *KE. Richkind*<sup>2</sup>. 1) Genzyme Genetics, Orange, CA; 2) Genzyme Genetics, Santa Fe, NM.

Interphase FISH analyses are routinely used to identify hyperdiploidy, MLL 11q23 rearrangements, t(12;21) and t(9;22) in childhood ALL. They may also identify additional abnormalities involving the loci tested. Results of 532 FISH analyses performed at Genzyme Genetics were reviewed to determine the frequency of such results and thereby determine the informativeness of interphase FISH studies, in addition to their intended purpose of identifying traditional abnormalities. 29 of 291 specimens tested for BCR/ABL showed abnormal results. 19 cases had BCR/ABL signals consistent with a t(9;22). Ten cases had the following abnormalities: additional copy of BCR/ABL[7], additional der(22)[1], ins(9;22)[1], deletion of der(9q)[1]. 48 of 164 specimens gave abnormal results with the 11q23 MLL probe. 30 cases had signals consistent with translocations involving 11q23. The remaining 18 cases had the following abnormalities: additional copy of MLL[9], one copy of MLL[5], rearrangement and deletion of MLL[2], rearrangement and additional MLL signal[1], 2 clones with two differing MLL abnormalities[1]. 47 of 138 specimens investigated for TEL/AML1 showed abnormal results. 26 cases had fusion signals consistent with t(12;21); the remaining 21 cases showed more complicated findings; gain of AML1[9], TEL/AML1 fusion with additional copy of TEL and/or TEL/AML1[5], TEL/AML1 and deletion of TEL[2], loss of TEL[3], two copies of TEL/AML1[1], reduced (split) AML1 signal[1]. Our results indicate 40% of cases, which are positive by FISH for BCR/ABL, TEL/AML1 or MLL rearrangements, will demonstrate additional or other changes involving these loci. Additionally, among cases with multiple FISH analyses, 2 cases with TEL/AML1 rearrangements also had deletion of MLL. Among cases with hyperdiploidy, 7 cases were also positive for TEL/AML1, and 8 had an abnormality involving MLL, including MLL rearrangement[2] and MLL deletion[1]. The prognostic implications of these additional abnormalities, in particular, a submicroscopic abnormality such as a deletion accompanying the traditional abnormality or the co-existence of two different traditional abnormalities, are yet to be determined.

**Modified allelic replication in genomes of patients with Beckwith Wiedemann Syndrome.** *O. Reish<sup>1</sup>, M.*

*Mashevitz<sup>1</sup>, R. Gobazov<sup>1</sup>, M. Rosenblat<sup>1</sup>, C. Sher<sup>1</sup>, V. Libman<sup>1</sup>, L. Avivi<sup>2</sup>.* 1) Genetic Inst, Assaf Harofeh Medical Ctr, Zerifin 70300 and Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; 2) Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv Israel.

Transcriptional activity of genes is related to their replication timing; alleles showing the common biallelic mode of expression replicate synchronously, whereas those with a monoallelic mode of expression replicate asynchronously. A loss of synchrony in allelic replication timing has been detected in lymphocytes derived from patients with various types of malignancies or premalignancies. As Beckwith Wiedemann Syndrome (BWS) is an overgrowth condition associated with an increased risk to develop malignancies, we used the fluorescence in situ hybridization (FISH) replication assay and evaluated the level of replication synchrony of three cancer-implicated genes (RB1, AML1, and CMYC) in lymphocytes derived from patients with BWS without malignancy. Ten controls and 6 patients with BWS including 2 sporadic individuals - one with paternal uniparental disomy (pat UPD), one with mosaic pat UPD and 4 individuals from the same family with enhancement of the paternal band at H19 locus, were evaluated. Karyotype analysis was normal in all patients. In cells derived from controls, each pair of alleles replicated synchronously; yet these same alleles replicated in a significantly increased asynchrony in cells derived from BWS patients. Each gene, which normally displayed synchrony in allelic replication, in the patients' cells displayed loss of synchrony. The loss of replication synchrony, of each gene, in the patients' cells was achieved by an advanced replication of a single allele, which replicated remarkably earlier than its normal scheduled timing. In addition, the second allele showed a significantly earlier replication timing than that normal for the gene. Thus, it is assumed that the BWS condition is associated with activation of cancer-implicated genes that may be the cause for over growth and an increased risk of patients to develop malignancies.

**FISH is a superior approach in the diagnostic confirmation of acute promyelocytic leukemia with PML/RARA insertions.** *G. Sun*<sup>1</sup>, *M. Owens*<sup>1</sup>, *H.Y. Dong*<sup>2</sup>, *W. Gorczyca*<sup>2</sup>, *S. Tugulea*<sup>2</sup>, *J. Weisberger*<sup>2</sup>. 1) Cytogenetics Department; 2) Division of Hematopathology, IMPATH Inc., New York, NY.

The presence of t(15;17) or the PML/RARA gene rearrangement is the hallmark of acute promyelocytic leukemia (APL). The introduction of all-trans retinoic acid (ATRA) to the chemotherapy regimen has dramatically improved clinical outcome. Given the potentially catastrophic clinical presentation and treatment stratification, a reliable and rapid diagnosis is essential. We compared the results of cytogenetics, FISH, and real-time multiplex PCR in 36 patients with APL (34 new diagnoses and 2 relapses). Cytogenetics was performed on 34 cases: there were 29 with t(15;17)(q22;q21) with or without additional chromosomal changes, 4 with a normal karyotype, and 1 with 47,XX,+8[5]/46XX[15]. FISH was performed on 12 of 36 cases using a PML/RARA dual color probe (Vysis), including all those without t(15;17). These 5 cases without t(15;17) showed unusual hybridization patterns in interphase and metaphase cells indicating insertions of a portion of the PML gene into the RARA gene (3 cases), and vice versa (2 cases). Therefore, patients suspected of having APL without a demonstrable t(15;17) may have an insertion of RARA or PML genes into chromosomes 15 or 17. APL with insertions comprised about 14% of all cases in our cohort. Nevertheless, cytogenetics has the advantage of detecting RARA gene rearrangements involving other chromosomes, such as t(11;17) and t(5;17), or additional chromosomal aberrations of potential prognostic importance. RT-PCR results from the cytogenetically cryptic 5 cases showed that 4 were positive for the long form (bcr1) transcript. One case with an insertion of the PML gene into the RARA gene was negative. Although RT-PCR is more sensitive for the detection of minimal residual disease and may provide prognostic information relating to specific fusion transcripts, these data show that FISH may detect rare cases of APL with breakpoints outside those typically detected by PCR, and also will detect a subset of APL cases with PML or RARA insertions other than the t(15;17) translocation.

**Breast cancer detection, treatment and outcome in women with hereditary breast and ovarian cancer (HBOC) - Alberta experience.** *R. Hughes<sup>1</sup>, B. Zochodne<sup>1</sup>, M. Lilley<sup>2</sup>, K. Blaikie<sup>1</sup>, S. Kieffer<sup>2</sup>, L. Wagter<sup>1</sup>, D. Gilchrist<sup>2</sup>, K. Barker<sup>1</sup>, G. Fick<sup>1</sup>.* 1) University of Calgary, Calgary, AB; 2) University of Alberta, Edmonton, AB.

Breast cancer detection and recurrence were evaluated in 41 women with BRCA gene mutations (mut) and 202 women with positive family histories (FHx) of hereditary breast and ovarian cancer (HBOC) from the Alberta Cancer Genetics Program. The average age at first breast cancer diagnosis was 42.3 yr +/- 9.0 yr SD in the mut group vs. 45.7 yr +/- in the FHx group. Right and left breasts were equally involved. The majority of breast cancers were first detected as palpable lumps (77.3% mut; 72.8% FHx) rather than by mammography (13.6% mut; 25.7% FHx). Many participants had mammograms done within the 2 years prior to breast cancer diagnosis that failed to detect the malignancies (37.5% mut; 51.5% FHx). Pathology review identified infiltrating ductal carcinomas (IDC) (93.9% mut; 88.5% FHx), infiltrating lobular carcinomas (ILC) (3.0% mut; 9.5% FHx), and mixed IDC/ILC (3.0% mut; 2.0% FHx). Choice of surgical treatment was divided between mastectomy (50% mut; 54.8% FHx) and segmentectomy/ lumpectomy (50% mut; 45.2% FHx). Subsequent breast ca diagnosis depended on initial surgical choice. mastectomy resulted in fewer recurrences and new primary diagnoses (17.6% mut; 16.5% FHx) compared to segmentectomy/ lumpectomy (41.2% mut; 27.1% FHx). Malignancies following mastectomy were more likely to occur in the contralateral breast (100% mut; 64.7% FHx) or lymph nodes/chest wall (0% mut; 35.3% FHx). Malignancies following seg/lumpectomy were more likely to occur in the ipsilateral breast (57.1% mut; 70.6% FHx). Conclusion: most breast cancers diagnosed in HBOC females are discovered by palpation. Mammograms performed within 2 years prior to diagnosis can miss tumours. Mastectomy reduces the risk of a second ipsilateral breast cancer diagnosis compared to seg/lumpectomy.

**Combining Clinical and Microarray Data for Tumor Classification.** *A. Smith<sup>1</sup>, J.M. Satagopan<sup>1</sup>, A. Stephenson<sup>2</sup>, M.W. Kattan<sup>1,2</sup>, W.L. Gerald<sup>3</sup>.* 1) Dept. of Epidemiology & Biostatistics; 2) Dept. of Urology; 3) Department of Pathology, Memorial Sloan-Kettering Cancer Ctr., New York NY.

A common objective of microarray analyses is the creation of a molecular model to classify samples into appropriate biological groups. As these studies are extremely expensive and laborious, this objective is only of value if the molecular classifier performs more reliably than existing clinical measures. There is increasing interest in the combining of clinical and molecular information to create a more robust classifier. Given an appropriate and reasonably large independent test set of samples, one can compare classification rates based on the clinical variable(s) alone, a molecular model alone, or a model which combines both. We propose a stepwise logistic regression procedure that will allow any combination of gene or clinical measures, without unduly weighting either. The procedure is demonstrated using a set of 79 prostate cancer tumor samples from Memorial Sloan-Kettering Cancer Center. The incidence of a biochemical recurrence was used to identify the response groups. Our results demonstrate that a combined model yields significantly improved classification over models based on a specific clinical measure of interest or gene expressions alone.

**Discovery and Classification of Cancer Types using Gene Expression Analysis.** *R.A. Alexandridis, S. Lin, M. Irwin.*  
Department of Statistics, Ohio State University, Columbus, OH.

Class discovery and classification are of crucial importance for determining efficient therapies in cancer treatment. Recent studies have shown that DNA microarray technology is a powerful tool for discovery and classification of cancer types and subtypes. We propose a unified approach to discovery of putative classes of cancers and classification of the interrogated tumor samples into the identified classes. Our approach is able to handle datasets with no training samples (class discovery problems), datasets where training samples exist and all the test samples belong to the known classes (classification), as well as datasets where training data exist but some of the test samples do not belong to any of the known classes (joint analysis of class discovery and classification). The method proposed is based on modeling the distribution of a gene expression profile as a finite mixture of an unknown number of distributions, with each mixture component characterizing the gene expression levels within a class. We use the expectation maximization algorithm (EM) to find the maximum likelihood estimates of the mixture model. Selection of the number of classes is achieved by Bayesian (BIC) or Akaike (AIC) information criteria. We reduce the large dimensionality by using several measures for gene selection, and explore the sensitivity of the class discovery and class prediction results to the gene selection measures and the number of selected genes. We applied our procedure to several datasets derived from the leukemia dataset of Golub et. al (1999) and to several simulation studies based on these datasets. For most leukemia datasets with 80 to 150 genes, the true number of classes is identified and the prediction accuracy rates are over 96% when there is not more than one unknown class. Even in the most difficult case, when there are three unknown classes and no training data, BIC discovers the true number of classes and the prediction accuracy rates are 89% or more for most datasets with 50 to 200 genes. The simulations further showed that our method is able to discover the true number of classes and to achieve good prediction accuracy.

**Most somatic mutations in human prostate cancer tissue conform to a specific motif.** *N. Makridakis, L. Ferraz, J.K.V. Reichardt.* Keck School of Medicine, Univ Southern California, Los Angeles, CA.

Cancer, including cancer of the prostate, is thought to progress through the accumulation of specific mutations in somatic tissue. We screened matched human prostate tumor and constitutional DNA for somatic mutations in three human genes, SRD5A2, HSD3B2 and HPRT, by direct sequencing of PCR products, in 30 patients. We identified a total of 85 somatic mutations in all three genes specifically in the tumor tissue, 84% of which are transitions. In contrast, only 55% of the constitutional DNA polymorphisms found in the same genes are transitions. In addition, we report that the sequence of 96% of the somatic mutations identified conforms to a nine nucleotide purine-rich motif. This motif is also found in all 26 H-ras gene mutations formed in the SENCAR mouse skin model, after induction of error-prone DNA repair less than one day following either benzopyrene or estradiol-3, 4-quinone treatment. 24 out of 26 (92%) of the H-ras gene mutations induced by either of these carcinogens in the mouse are also transitions. Literature searches identified the same purine-rich motif in 76 out of 80 (95%) of the somatic prostate cancer mutations of all the genes analyzed in humans, including H-ras, N-ras, Ki-ras, PTEN, and the androgen receptor gene. These data suggest that the majority of the somatic mutations in human prostate cancer tissue are transitions that occur in a specific purine-rich motif, which may be the target of a particular pathway of error-prone DNA repair.

**Replication and reproducibility in microarray experiments testing chemotherapeutic effects in an animal model.**

*W. Liu*<sup>1</sup>, *G.A. Chase*<sup>1</sup>, *W.B. Reeves*<sup>2</sup>, *X. Gao*<sup>2</sup>. 1) Department of Health Evaluation Sciences, Penn State College of Medicine, Hershey, PA 17033; 2) Department of Nephrology, Penn State College of Medicine, Hershey, PA 17033.

DNA microarray experiments measure the expression of thousands of genes simultaneously but at a high cost. One major problem facing many small labs is that of setting a minimal number of replicates for a reproducible result. Here we studied the performance of statistical significance tests for different replication schemes by analyzing two independent experiments. In our first experiment, gene expression alterations in kidney between wild type and TNF alpha knockout mice under the influence of cisplatin, a popular drug used in cancer treatment, were studied. We tested genes for significant changes in expression using permutation tests implemented by the computer software SAM. The sensitivities and false discovery rates (FDR) of the tests using four, three, two and one pair of dye-swapped replicate arrays were compared. Sensitivities were calculated with respect to the results obtained using four pairs of replicates as a gold standard. Sensitivity decreases with fewer replicates and FDR increases with fewer replicates. The overall average sensitivity of the tests using three pairs of replicates was relatively high (.89) and the average FDR was low (.034). However, the sensitivities and false discovery rates of the tests using two and one pair of replicates were unsatisfactory, especially for tests using only one pair of replicates. To verify our results we compared gene expression differences in kidney tissue between cisplatin treated and untreated mice; similar results were found. In conclusion, our results indicate that the use of one or two pairs of replicates leads to too many false positive genes and a low power to detect truly significant gene changes. We suggest the use of at least three pairs of replicates to obtain a reproducible result.

Program Nr: 342 from 2003 ASHG Annual Meeting

**CGAP and MGC - two comprehensive genomic resources for the scientific research community.** *L. Jia, D. Gerhard, L. Grouse, S. Greenhut.* Office of Cancer Genomics, National Cancer Institute, and the CGAP and MGC Project Teams, NIH, Bethesda, MD.

The NCI's Cancer Genome Anatomy Project (CGAP, <http://cgap.nci.nih.gov/>) provides a web-based information platform to study the gene expression profiles of normal, precancer, and cancer cells, leading eventually to improved detection, diagnosis, and treatment for the patient. Using CGAP's web analysis tools, researchers can examine gene expression in human and mouse tissues using either EST or SAGE data, search for SNPs, find chromosome aberrations in cancer, identify FISH-mapped BACs or cDNA clones that are useful in their research, and explore genes and their pathways. CGAP continues to add to its scientific expertise and expand its databases for the benefit of all cancer researchers. The NIH's Mammalian Gene Collection (MGC, <http://mgc.nci.nih.gov/>) provides full-length open reading frame (FL-ORF) clones for human and mouse genes. Using standard, normalization, and size-selected methods, the MGC has produced full-length cDNA libraries from over 100 human tissues and cell lines and 80 mouse tissues. To date, clones for a non-redundant set of more than 15,000 human genes and 11,000 mouse genes have been sequenced and verified to contain complete FL-ORFs. All MGC sequences are deposited in GenBank and the clones can be purchased from distributors of the IMAGE Consortium.

**Tumor classification using phylogenetic methods on expression data.** *A.A. Schaffer<sup>1</sup>, R. Desper<sup>1</sup>, J. Khan<sup>2</sup>*. 1) NCBI/NIH/DHHS, Bethesda, MD; 2) NCI/NIH/DHHS, Gaithersburg, MD.

Tumor classification is a well-studied problem in bioinformatics. Developments in the field of DNA chip design have now made it possible to measure the expression levels of thousands of genes in sample tissue from healthy cell lines or tumors. A number of studies have examined the problem of tumor classification: using the data from a DNA chip to accurately classify an unknown tumor. This problem is of high interest whether one seeks to differentiate cancerous tissue from healthy tissue, or whether one seeks to accurately classify unknown tumors according to a diagnostic or prognostic category. Our work applies phylogenetic methods to this problem. We impose a metric on a set of tumors as a function of their gene expression levels, and we seek to infer a tree structure from the distance data, using tree fitting methods borrowed from the field of phylogenetics. Phylogenetic methods provide a simple way of imposing a hierarchical relationship on the data, with branch lengths in a phylogeny representing a degree of separation. We demonstrate the flexibility and robustness of the phylogenetic method with regard to resampling methods such as jackknifing and with regard to noise perturbation of the real data. Using our methods on a published data set of 87 tissues, comprised mostly of small, round, blue-cell tumors (SRBCTs), we fit the 87 samples to a pseudo-phylogenetic tree, which neatly separated into 4 major clusters corresponding exactly to the four groups of tumors: neuroblastomas, rhabdomyosarcomas, Burkitt's lymphomas, and Ewing's sarcomas. We also tested our methods on a published data set of 22 breast tumors. The resulting tree separated tumors with BRCA1 mutations from those with BRCA2 mutations, with sporadic tumors separated from both other groups and from each other.

**Differential reduction of qkI isoforms in human glioma cell lines.** *L. Ku, Y. Feng.* Emory University, Department of Pharmacology, Atlanta, GA b.

Cytogenetic alterations at 6q25-26 has been reported to associate with a variety of human malignancies, including gliomas. However, which gene(s) at this locus may play a role as potential tumor suppressor mains unclear. The human quaking gene (Hqk) has been recently mapped to 6q25-26, which encodes a selective RNA-binding protein QKI, a member of the signal transduction activators of RNA (STAR). Three major isoforms of QKI are derived from alternative splicing of the qkI primary transcript, which are named QKI-5, 6 and7 based on the length of the corresponding mRNA. The role of QKI has been implicated in cell growth, differentiation, as well as apoptosis. In the brain, all QKI isoforms are expressed in various types of glial cells but absent in neurons. We analyzed qkI mRNA expression in 23 glioma cell lines. Our preliminary result indicated that around one third of the tumor lines showed significant reduction of the total qkI transcripts. Interestingly, qkI-7, the isoform that can act as a potent apoptosis inducer, was preferentially reduced. Moreover, qkI-6, the isoform that promotes glia differentiation was diminished in most of the glioma lines analyzed. In contrast, qkI-5, the embryonic predominant isoform remains normal in all the glioma cell lines analyzed. These results suggest that abnormalities of qkI alternative splicing may affect glial differentiation and apoptosis, potentially in turn contributes to glioma tumorigenesis.

**Increased chromosomal instability at common fragile sites in Seckel syndrome.** *S.G. Durkin, A.M. Casper, T.W. Glover.* Department of Human Genetics, University of Michigan, Ann Arbor, MI.

The partial perturbation of DNA replication induces the expression of common fragile sites. These sites are detected as gaps and breaks on metaphase chromosomes under conditions of replicative stress, such as from aphidicolin treatment or folate deficiency. Rather than arising by mutation, common fragile sites are a constant component of chromosome structure. These sites are often rearranged in tumor cells, and thus are important for understanding chromosomal instability observed in cancer. We have previously shown that ATR, a gene critical to S phase and G2/M checkpoint signaling in response to stalled replication forks, is crucial for the maintenance of chromosomal stability at common fragile sites. Cells lacking ATR are non-viable, making the effects of ATR deficiency challenging to study. Recently, however, a subgroup of patients with Seckel syndrome was reported by O'Driscoll et al. (*Nat. Genet.* 33:497-501, 2003) to have a mutation in ATR. Seckel syndrome is a heterogeneous disorder characterized by severe dwarfism, mental retardation, microcephaly, and in some cases, chromosome instability, hematological disorders and leukemia. Two Pakistani families with Seckel syndrome were found to have a silent mutation in ATR that resulted in use of cryptic splice donor sites in exon 9, leading to a frameshift. Homozygotes for this hypomorphic allele produce greatly reduced levels of correctly spliced message. We hypothesized that cells from Seckel syndrome patients would have increased instability at common fragile sites. We obtained lymphoblastoid cell lines from two affected and three unaffected members of these families. ATR deficiency in all affected individuals was confirmed by western blots. Following treatment with aphidicolin, cells homozygous for the hypomorphic allele showed a ~3 fold increase in total gaps and breaks, and in breaks at specific fragile sites, compared to unaffected controls. These results are consistent with our previous findings that ATR deficiency results in increased fragile site instability and suggest that the Seckel syndrome hypomorphic mutation provides a model for studying the biological effects of fragile site instability.

**The Association of Up-regulation of FGF3 and Hepatocellular Carcinoma Metastasis and Recurrence. X-Y.**

*Guan<sup>1</sup>, L. Hu<sup>1</sup>, D. Xie<sup>1</sup>, J.S. Sham<sup>1</sup>, J-M. Wen<sup>2</sup>, W-S. Wang<sup>3</sup>.* 1) Department Clinical Oncology, University Hong Kong, Hong Kong, China; 2) Department Pathology, Sun Yat-Sen University, Guangzhou, China; 3) Department Surgery, First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China.

Hepatocellular Carcinoma (HCC) is one of the most common malignancies worldwide with poor prognosis. The poor prognosis of HCC has been associated with the tumor metastasis and recurrence. Therefore, it is imperative to completely understand the molecular mechanisms involved in the tumor metastasis in HCC. 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 chromosome microdissection. The result showed that the HSR was composed of DNA sequence from 11q13. Amplification and overexpression status of CCND1-FGF19-FGF4-FGF3 gene cluster in 11q13 in H4-M was analyzed by Southern blot and Northern blot hybridizations. Amplification and overexpression of CCND1 and FGF3 were detected. The association of overexpression of FGF3 and HCC metastasis as well as recurrence was studied using a tissue microarray composed of 60 pairs of primary and matched metastatic HCCs and 30 pairs of primary and matched recurrent HCCs. The results showed that the overexpression frequency of FGF3 was significantly higher in metastatic HCC (19/40, 47.5%) than that in primary HCC (3/40, 7.5%). Similarly, the frequency of FGF3 overexpression was significantly higher in recurrent HCC (9/21, 42.86%) than that in primary HCC (1/21, 4.8%). Our results strongly suggested that up-regulation of FGF3 may play an important role in metastasis and recurrence of HCC.

**Altered Notch signaling resulting from expression of a WAMTP1-MAML2 gene fusion in mucoepidermoid carcinomas.** *F. Enlund<sup>1</sup>, A. Behboudi<sup>1</sup>, Y. Andren<sup>1</sup>, C. Öberg<sup>2</sup>, U. Lendahl<sup>2</sup>, J. Mark<sup>1</sup>, G. Stenman<sup>1</sup>.* 1) Lundberg Laboratory of Cancer Research, Dept of Pathology, Univ. Gothenburg, Sweden; 2) Dept of Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden.

Chromosome translocations in neoplasia commonly result in fusion genes that may encode either novel fusion proteins or normal, but ectopically expressed, proteins. Here we report the cloning of a novel fusion gene in a common type of salivary and bronchial gland tumor, mucoepidermoid carcinoma (MEC). The fusion, which results from a t(11;19)(q21-22;p13) translocation, creates a chimeric gene in which exon 1 of a novel gene of unknown function, designated WAMTP1, is linked to exons 2-5 of the recently identified Mastermind-like Notch coactivator MAML2. In the fusion protein, the N-terminal basic domain of MAML2, which is required for binding to intracellular Notch (ICD), is replaced by an unrelated N-terminal sequence from WAMTP1. Northern blot analyses revealed that the expression of WAMTP1 is restricted to certain fetal and adult tissues while MAML2 is expressed in most tissues. The fusion protein was found to colocalize with both MAML2 and Notch1 ICD to nuclear granules. Analysis of Notch target genes revealed altered expression of several genes in fusion positive MECs compared to normal salivary gland tissue and MECs lacking the fusion. These findings suggest that altered Notch signaling plays an important role in the genesis of neoplasms of salivary and bronchial gland origin.

**Differential Expression of a Gonadoblastoma Candidate Gene in Gonadoblastoma, Testis and Prostate Cancer Suggests a Role of the Y Chromosome in Human Oncogenesis.** *Y.-F.C. Lau, H. Lau, Y. Li, L.G. Komuves.* Dept Medicine/ VA Med Ctr/ Univ California, San Francisco, CA.

Gonadoblastoma on the Y chromosome (GBY) is an oncogenic locus that predisposes the dysgenetic gonads of XY sex-reversed females to tumorigenesis at high frequency. Recent completion of the human Y chromosome sequencing revealed the testis-specific protein Y-encoded (TSPY) gene as the only functional gene in the GBY critical region. TSPY is 2.8-kb in size and is repeated tandemly in 20.5-kb units. TSPY shares significant homology to various cyclin B binding proteins, including oncoprotein SET, some of which possess cell cycle modulating functions. TSPY is expressed at high levels in both gonadoblastoma and testicular seminoma. To further evaluate the role of TSPY in other male-specific cancers, we had examined its expression in 10 cases of prostate cancer using both in situ hybridization and immunohistochemistry. Our results demonstrated that TSPY was expressed at low levels in normal epithelial cells and benign prostatic hyperplasia, but at elevated levels in tumor cells of prostate cancer at various degrees of malignancy. Sequence analysis of RT-PCR products obtained from both prostatic and testicular tissues using primers flanking the ORF revealed a complex pattern of RNA processing of the TSPY transcripts involving cryptic intron splicing and/or intron skipping. All TSPY transcripts maintain the same ORF and encode a variety of polymorphic or shortened versions of TSPY with diverging properties ranging from 18.1 to 35.1 kDa in size and pI from 5 to 9. The abbreviated TSPY transcripts were more abundant in prostatic cancer than testis samples. GST pull-down assays demonstrated that all TSPY proteins were capable of binding to cyclin B. The binding domain was mapped to their carboxyl termini that share the most significant homology to the oncoprotein SET. Their preferential expression in cancer, diverse biochemical properties and conserved cyclin B binding function suggest that the TSPY gene/variants may be involved in the multi-step prostatic oncogenesis beside its putative oncogenic or tumor-promoting role in gonadoblastoma and testicular seminoma.

**Cancer risk assessment in Indian oral precancer patients using SCGE assay and micronucleus test.** *R. Shukla<sup>1</sup>, P.P. Reddy<sup>2</sup>, Y.R. Ahuja<sup>3</sup>*. 1) Genetic Unit, Dept.Pediatrics, AIIMS, New Delhi, India; 2) Inst of Genetics, Begumpet, Hyderabad, India; 3) Vasavi Med and Res Ctr, 6-1-91, Khairtabad, Hyderabad, India.

Carcinoma of the oral cavity is the most common malignancy in the Indian subcontinent constituting 9.8% of all cancer cases. In India, oral cancer has been causally associated with chewing of tobacco with or without betel quid, which increases exposure to carcinogenic tobacco specific nitrosamines (TSNA) and nitrosamines derived from areca nut alkaloids. An early sign of damage to the oral mucosa is the development of well-defined precancers like leukoplakia, erythroplakia, lichen planus, and submucous fibrosis. While some of the precancers regress, about one third of these may progress to invasive malignancy over a period of time. Thus, there is an urgent need for biomarkers that can predict whether a precancer will develop into an aggressive or metastasizing tumor. This study, using the micronucleus test (MNT) and single cell gel electrophoresis (SCGE) assay was planned with an attempt to understand the carcinogenic process in oral cancer, particularly with reference to DNA damage. The aim was to develop a risk index, for oral precancer patients, based on genomic damage and chromosome instability, to identify those precancer patients who are at high risk for malignant transformation. MNT and SCGE were performed on the buccal epithelial cells (BEC) and peripheral blood leukocytes (PBL) of 443 subjects (129 oral cancer patients, 138 precancer patients and 176 controls) to assess basal DNA damage, MNNG sensitivity and DNA repair. A high-risk precancer patient was identified on the basis of 4 criteria (1) high basal DNA damage in both PBL and BEC (using SCGE) (2) high incidence of chromosome damage in BEC (using MNT) (3) increased sensitivity to MNNG and (4) reduced repair capacity (using SCGE). Based on the above criteria, 4 of 138 oral precancer patients were identified as being at high risk. On follow-up, 3 of these 4 precancer patients presented with malignant lesions after about two years of initial diagnosis. This finding suggests that simple, rapid and sensitive assays such as MNT and SCGE can be included in the battery of tests used for cancer risk prediction.

**An in vitro model for human papillomavirus integration in cervical cancer.** *T. Yu, D.I. Smith.* Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

Human papillomaviruses (HPVs) are the major causative agent responsible for the development of cervical cancer (CC). High-risk HPVs can efficiently immortalize cells but additional alterations are necessary to transform those cells and cause the development of invasive CC. The development of invasive CC is temporally associated with the integration of HPV sequences into the host genome. An analysis of the sites of HPV integration has revealed that integration occurs throughout the genome. This has led to the currently accepted dogma that the site of HPV integration is unimportant for the eventual CC that develops. We have been analyzing the sites of HPV16 and HPV18 integration in CC and have found that integration sites are non-random. Over half of the viral integrations occur into common fragile sites (CFS), and some regions have multiple HPV integrations, including the FHIT gene, and the region surrounding c-myc. To determine whether the sites of HPV integration play an important role in CC development we have developed an in vitro model of HPV integration. Linearized DNA will preferentially integrate into CFS regions when cells are exposed to aphidicolin. We utilized this to target the integration of HPV16 and HPV18 DNA sequences into CFS regions. We took human foreskin keratinocytes and exposed them to aphidicolin. We then electroporated an HPV16 and an HPV18 cassette (with a neomycin selectable marker) into these keratinocytes followed by G418 selection. This procedure generated large numbers of G418 resistant colonies that contained HPV sequences integrated into the keratinocytes. We have begun to analyze the sites of HPV integration using the powerful technique of restriction site oligonucleotide PCR (the technique that we previously utilized to map HPV16 and HPV18 integrations in primary cervical tumors). We have found that the HPV16 and HPV18 cassettes preferentially integrate into CFS regions. This in vitro system will enable us to compare the effect of HPV integration into CFS regions that occur in vivo in cervical tumors to integration in the exact same regions in vitro. This should enable us to determine if the sites of HPV integration play an important role in the eventual CCs that develop.

**Genomic disorganization in HCC3153, a new BRCA1 deficient breast tumor cell line.** *G.E. Tomlinson, T. Chen, N.R. Schneider, L. Girard, A. Virmani, J.D. Minna, A. Gazdar.* Dept Ped, Hamon Ctr Ther Onc, Univ TX Southwestern Med Ctr, Dallas, TX.

BRCA1 is a tumor suppressor known to be associated with a high susceptibility to breast cancer. This susceptibility is now thought to be due to a basic defect in maintaining genomic stability. Numerous studies have now documented a role of BRCA1 in nucleotide excision repair and double strand break repair. Few studies have reported changes in human BRCA1 deficient breast tumor cells at the chromosomal level. We previously described genetic changes in a BRCA1 cell line, HCC1937. We report here a new tumor cell line, HHCC3153, derived from a BRCA1 mutation carrier with a germline BRCA1-943ins10 mutation. This mutation is known to be an African American founder mutation. The cell line lacks a normal copy of BRCA1 and is also homozygous for an acquired deletion in exon 4 of the TP53 gene resulting in a protein truncation.

Karyotyping of cultured tumor cells was performed using standard methods. These cultured cells are characterized by extreme aneuploidy. No two metaphases demonstrated an identical pattern. Two overlapping composite karyotypes were constructed, one with 56-59 chromosomes and one with 125 - 161 chromosomes. Many unidentifiable chromosomal fragments were observed. There were no normal chromosomes recognized.

A genome wide survey of allelic loss using 399 fluorescent markers was also performed. The fraction of alleles which demonstrated allele loss was .34 which is moderately high, but not outside the range we usually observe in cultured breast cell lines.

Our findings suggest that in addition to playing a role in transcription coupled repair, that there is an effect of BRCA1 loss at the chromosomal level .

**Familial Melanoma, pancreatic cancer and germline CDKN2A mutations.** *A.M. Goldstein.* Genetic Epidemiology Branch, DCEG, National Cancer Institute, NIH, DHHS, Bethesda, MD.

The CDKN2A gene is the major identified high-risk melanoma susceptibility gene. CDKN2A encodes two cell-cycle regulatory proteins, p16 and p14ARF. Germline CDKN2A mutations have been observed in approximately 20 percent of familial melanoma kindreds from North America, Europe and Australasia. There is also an increased risk of pancreatic cancer in a subset of families with mutations, however, the precise relationship between the CDKN2A gene and pancreatic cancer remains unknown. This study used published data to examine the relationships between familial melanoma, pancreatic cancer and germline CDKN2A mutations. The types and frequencies of the mutations, their distributions throughout the gene, and evidence for identifiable patterns were determined. Nonparametric statistics were used to test the hypotheses of no differences in the distribution of CDKN2A mutations or families with CDKN2A mutations for the factors of interest. Overall, there were 67 different CDKN2A mutations observed in 188 melanoma-prone families. The most common types of mutations were missense (55 percent), frameshift (12 percent), and splice site (9 percent). About two-thirds of the mutations were observed only once, while the remainder recurred in different families. Comparison of all 188 melanoma-prone families to the 42 families that also contained pancreatic cancer showed no significant differences in the types of mutations ( $p=0.97$ ) or mutation location across exons ( $p=0.78$ ) or the four ankyrin (Ank) repeats ( $p=0.27$ ). There was also no significant difference whether the mutations ( $p=0.61$ ) or families ( $p=0.49$ ) altered p14ARF function. However, there was a significant difference ( $p=0.02$ ) in the numbers of families with mutations across the four ankyrin repeats with a deficit of families with CDKN2A mutations with pancreatic cancer in Ank1 and Ank2 (9 percent in Ank1-2 versus 34 percent in Ank3-4). Further research utilizing individual-specific data will be required to determine whether these patterns represent etiologic differences or incomplete reporting of cancer and mutation data.

**BRCA1-related breast cancer is characterized by a basal epithelial phenotype.** *W.D. Foulkes<sup>1</sup>, I.M. Stefansson<sup>2</sup>, P.O. Chappuis<sup>1</sup>, L.R. Bégin<sup>1</sup>, N. Wong<sup>1</sup>, M. Trudel<sup>1</sup>, L.A. Akslen<sup>2</sup>.* 1) Program in Cancer Genetics and Departments of Oncology, Human Genetics, Medicine, Surgery and Pathology; Research Institute of the McGill University Health Centre; Cancer Prevention Centre, Sir M.B. Davis-Jewish General Hospital; McGill University, Montreal, Quebec, Canada; 2) Department of Pathology, The Gade Institute, Haukeland University Hospital, N-5021 Bergen, Norway.

A basal epithelial phenotype accounts for 15% of all invasive breast cancers. Microarray studies have shown that this phenotype is characteristic of breast cancers that express neither estrogen receptor (ER) nor erbB-2 (HER2/neu). As the ER/erbB-2 negative phenotype is also characteristic of BRCA1-related breast cancer, we hypothesized that BRCA1-related breast cancer would be more likely than non-hereditary cancer to express basal keratins. Among 292 breast cancers previously analysed for ER, erbB-2, p53 and germ-line BRCA1/2 mutations, we identified 76 tumors that did not over-express ER or erbB-2. In the 72 cases with sufficient material, there were 17 BRCA1 carriers and 55 BRCA1/2 non-carriers. Forty tumors (56%) stained positively for a mouse monoclonal antibody raised against cytokeratin 5/6 (CK5/6).

The presence of CK5/6 staining was associated with high grade, lymph node negative tumors, and expression of p53. Of the 17 BRCA1-related tumors that were ER and erbB-2 negative and for whom CK5/6 results were available, 15 (88%) over-expressed CK5/6. Among non-BRCA1/2 carriers who had an ER/erbB-2 negative phenotype, only 25/55 (45%) expressed CK5/6 (odds ratio 9.0, 95% CI: 1.9-43, P = .002). These findings suggest that germ-line BRCA1 mutations are associated with a distinctive breast cancer phenotype.

Breast stem cells in rodents and humans can have a CK5/6 + profile. The pattern of genetic alterations identified in all CK5/6 + breast cancers are very similar to those observed in BRCA1-related breast cancers. Therefore, a role for BRCA1 in determining the cytokeratin profile of breast cancer, via regulation of breast stem cells is plausible.

**hMLH1 and hMSH2 mutations in Colombian families with HNPCC. Description of two novel mutations. A.**

*Giraldo<sup>1,2</sup>, A. Gomez<sup>1</sup>, A. Salguero<sup>1</sup>, H. Garcia<sup>1</sup>, F. Aristizabal<sup>1</sup>, O. Gutierrez<sup>1</sup>, L.A. Angel<sup>1</sup>, J. Padron<sup>3</sup>, C. Martinez<sup>4</sup>, H. Martinez<sup>1</sup>, O. Malaver<sup>1</sup>, L. Florez<sup>5</sup>, R. Barvo<sup>6</sup>.* 1) Instituto de Genetica & Facultad de Medicina, Universidad Nacional de Colombia, Bogota; 2) Fundacion Gillow; 3) Univ. El Rosario; 4) Hosp. Militar; 5) Hosp. San Jose. Bogota; 6) Clin. la Concepcion, Barranquilla.

In Colombia, colorectal cancer (CCR) is the fourth cause of death among different cancers. From 3 to 5% of all CCR correspond to Hereditary Nonpolyposis Colorectal carcinoma (HNPCC) or Lynch syndrome. hMLH1 and hMSH2 genes are the most frequent genes involved in HNPCC pathogenesis. We present the results observed in 17 families fulfilling Amsterdam II, or Bethesda criteria. 35 exons of hMLH1 and hMSH2 were screened by SSCP and all electrophoretic variants were automatically sequenced. We detected six germinal mutations in the analyzed families, five in the hMLH1 and one in the hMSH2, which corresponds to 35%. When analyzing the 6 families fulfilling the Amsterdam criteria we detected 83% of the mutations. Two apparently non related families, of the same geographical region share the same mutation, which suggest a founder effect. Three of the five different observed mutations have previously been reported in the International Collaborative Group Database or in the Human Gene Mutation Database. The previous reported mutation of hMLH1 gene are, a single base substitution at the donor splicing site of exon 9, a single base substitution (A/G) at codon 755 of the exon 17, and another single base substitution (G/A) at codon 681 of exon 18. The novel mutations are, a single base substitution (C/T) at codon 640 of exon 17 of the hMLH1 gene and a two nucleotide deletion (TG) at codon 184 of exon 3 of hMSH2 gene. Also, in two families a polymorphism in the intron 13 (G/A) nt 1558+14, of hMLH1 gene was detected. This polymorphism is present in 6.5% of a sample of 150 Colombian individuals. In the present study, most of the mutations, are in the hMLH1 gene which similar to that observed in other Latin American studies. This is the first study performed in Colombia and pretends to establish a prevention program.

**Familial aggregation of lymphoproliferative tumors.** *L.R. Goldin*<sup>1</sup>, *R.M. Pfeiffer*<sup>2</sup>, *M.H. Gail*<sup>2</sup>, *L. Møller*<sup>3</sup>, *J.H. Olsen*<sup>3</sup>, *K. Hemminki*<sup>4</sup>. 1) Gen Epidemiol Br, DCEG/NCI, Bethesda, MD; 2) Biostat Br, DCEG/NCI, Bethesda, MD; 3) Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark; 4) Dept. of Biosciences at Novum, Karolinska Institute, Stockholm, Sweden.

Many case studies and population or hospital-based surveys have reported familial aggregations of Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL), chronic lymphocytic leukemia (CLL) and multiple myeloma (MM). To quantify the familial aggregation among these lymphoproliferative (LP) tumor types, we used two large family cancer databases from Sweden and Denmark that were created by linking population registries (containing parent-offspring links) to cancer registries. We have cancer outcome data on first-degree relatives of approximately 52,000 cases of HL, NHL, CLL, or MM and on relatives from matched control groups. We used a marginal survival model comparing disease occurrence in relatives of case compared to control probands. A robust variance estimate accounts for dependencies among relatives. Testing familial aggregation corresponds to testing the null hypothesis that the hazard ratio (HR) of being a relative of a case proband compared to a control proband is 1.0. Relatives of HL cases are at significantly increased risk for HL with a HR = 3.5 in Sweden ( $p < .0001$ ) and HR=2.5 in Denmark ( $p = .03$ ) and aggregation is strongest in males, in siblings, and in relatives of early onset cases. For example, the HR in the combined samples for relatives of probands with age of diagnosis 40 and under is 3.9 ( $p < .0001$ ) compared to 1.6 (ns) for relatives of probands with age of diagnosis greater than 40. In the Swedish sample, relatives of CLL cases are at very high risk for CLL (HR=7.5,  $p < .0001$ ) and elevated risk of HL (HR=2.3,  $p = .03$ ) and NHL (HR=1.4,  $p = .06$ ), relatives of NHL patients are at increased risk for NHL (HR=1.8,  $p < .0001$ ) and HL (HR=1.5,  $p = .04$ ) and relatives of MM patients are at increased risk for MM only (HR=1.7,  $p = .04$ ). We conclude that relatives have the highest risk for developing the same LP tumor as the proband, but there are also significantly elevated risks for other related LP tumors. This suggests shared genetic etiology, especially among HL, NHL, and CLL.

**Analysis of ZBRK1 polymorphisms in high-risk non-*BRCA1/2* French-Canadian families.** *S. Desjardins, Y. Labrie, G. Ouellette, J. Simard, INHERIT BRCA's, F. Durocher.* Cancer Genomics Laboratory, CHUL Research Ctr, CHUQ, Laval University Quebec, Canada.

ZBRK1 is a BRCA1-interacting protein with an N-terminal KRAB domain and a central motif containing eight zinc fingers involved in the recognition and negative regulation of *GADD45* expression. This BRCA1-mediated repression of *GADD45* by *ZBRK1* plays an important role in cell-cycle regulation following DNA damages as well as in maintenance of genome stability. To determine the possible influence of *ZBRK1* polymorphisms on breast cancer susceptibility in non-*BRCA1/2* families, we studied the coding sequence of the *ZBRK1* gene in approximately 70 individuals from 58 high-risk French-Canadian families in which no mutation in either *BRCA1* or *BRCA2* has been identified. We analyzed 12 sequence variants, among which a new non-conservative change, R132C, was identified while another one is in the 3'UTR region. The 5 silent mutations and 6 missense mutations analyzed show similar frequencies to those reported by Struewing et al. in a North American population. Four polymorphisms are very rare (frequencies ranging from 0.01 to 0.04: I69T, R132C, S476S and V524I), 5 have a frequency between 0.05 and 0.10: C236C, P373P, T385T, S472P and S501R, and 3 display a frequency which is greater than 0.10: N35N, P66L and 1672C/T. The most frequent coding polymorphisms are located within the KRAB domain (N35N: 0.25, P66L: 0.18), a region needed for the repressor activity of ZBRK1 in concert with the BRCA-binding domain (amino acids 319 to 532), in which 6 other polymorphisms are situated. Another important region of the *ZBRK1* gene consists of eight zinc finger motifs implicated in DNA sequence recognition. Three different variations (C236C, P373P and T385T) fall within this domain. Among the non-*BRCA1/2* individuals studied, we have found 18 different combinations of these 12 polymorphisms. 79% of individuals (53/67) have one of the 4 commonest combinations, while the remaining 21% (14 individuals) seem to have a unique arrangement. There is strong linkage disequilibrium between these polymorphisms, even between the two furthest variations (N35N and 1672C/T) which are located approximately 4260 bp apart.

**Clinical response to induction chemotherapy or radiotherapy related to BRCA1 and BRCA2 mutations in familial invasive breast cancer.** *I. Coupier<sup>1</sup>, D. Stoppa-Lyonnet<sup>1</sup>, B. Sigal-Zafrani<sup>2</sup>, A. Renard<sup>3</sup>, P. Pouillart<sup>4</sup>, K.B. Clough<sup>5</sup>, B. Asselain<sup>6</sup>, A. Fourquet for the Institut Curie Breast Cancer Group<sup>3</sup>.* 1) Department of Genetics, Institut Curie, Paris, France; 2) Department of Pathology, Institut Curie; 3) Department of Radiation Oncology, Institut Curie; 4) Department of Medical Oncology, Institut Curie; 5) Department of Surgery, Institut Curie; 6) Department of Biostatistics, Institut Curie.

**Purpose:** BRCA1 BRCA2 germline mutations are associated with impaired DNA double-strand break repair. We tested whether breast cancers in BRCA mutation carriers were more responsive to induction neoadjuvant treatments than in non-carriers. **Methods:** BRCA1 and BRCA2 mutations were screened by DGGE or DHPLC through their coding sequence in a retrospective cohort of 91 patients (with 94 tumors) with a family history of breast and / or ovarian cancer, treated with induction anthracycline-containing chemotherapy and /or neoadjuvant radiotherapy. Clinical responses and breast preservation rates were correlated to BRCA1 status, and to other clinical and pathologic factors. **Results:** Following chemotherapy, a complete response (CR) was obtained in 11/24 BRCA1 tumors (46%) and in 11/51 non BRCA1 tumors (22%)( $p=0.003$ ). A major response was observed in 20/24 (83%) and 36/51 (70.5%), respectively. Following radiotherapy, a major response was seen in 5/5, and 8/14, respectively (CR: 1/5, and 3/14). Breast conservation was possible in 25/29 (86%)BRCA1 tumors and 41/65 (63%) non BRCA1 tumors ( $p=0.02$ ). BRCA1 mutation, high mitotic count, lack of estrogen receptor expression and tumor size predicted response to induction treatments. However, BRCA1 mutation was the sole predictor of breast conservation. BRCA2 analysis is still in progress. **Conclusion:** Breast conservation after induction treatment was higher in BRCA1 carriers, and clinical response was related to aggressive tumor features correlated with BRCA1 mutations. This suggests that impaired DNA repair related to BRCA1 mutations increased chemosensitivity and radiosensitivity of breast cancer. Further studies are needed to determine the long-term local outcome of these patients.

**Genomic rearrangements in *MSH2* and *MLH1* are rare mutational events in Spanish hereditary nonpolyposis colorectal cancer patients.** *S. Castellví-Bel*<sup>1</sup>, *V. Piñol*<sup>1</sup>, *M. Milà*<sup>2</sup>, *J.M. Piqué*<sup>1</sup>, *A. Castells*<sup>1</sup>. 1) Gastroenterology, Hospital Clinic, Barcelona, Spain; 2) Genetics, Hospital Clinic, Barcelona, Spain.

Hereditary nonpolyposis colorectal cancer (HNPCC) is the most frequent autosomal dominant predisposition to the development of colorectal cancer (CRC). It is caused by mutations in one of the DNA mismatch repair (MMR) genes, the majority of which occur in *MSH2* and *MLH1*. Recently, genomic rearrangements in the *MSH2* and *MLH1* genes have been reported to account for an important proportion of the mutation spectrum in these MMR genes, making advisable to begin the molecular analysis of *MSH2* and *MLH1* by screening for such rearrangements.

Patients and methods: As part of a population-based, multicenter study aimed at determining HNPCC frequency in Spain, 1,025 tumor and non-tumor paired DNA samples from CRC patients were collected in an one-year period. Patients were clinically characterized and molecular studies (microsatellite instability (MSI) and MSH2/MLH1 immunohistochemistry) were performed in all of them. Patients whose tumor showed MSI or did not express MSH2/MLH1 (n=100) were selected to undergo further molecular analyses (genomic rearrangements in the *MSH2* and *MLH1* genes and sequencing). Furthermore, 20 additional Amsterdam-positive HNPCC patients referred to our center for genetic analysis were also included. Genomic rearrangements were analyzed using the MLPA (multiplex ligation-dependent probe amplification) technology before undergoing sequencing analysis of the *MSH2* and *MLH1* genes. Control DNA samples with known genomic rearrangements in *MSH2* or *MLH1* were included.

Results: MLPA did not detect any genomic rearrangements in our study population, which included patients with extensive MMR defects, both positive and negative for HNPCC-Amsterdam II criteria. Alterations in either *MSH2* or *MLH1* were clearly detected in control DNA samples.

Conclusions: Genomic rearrangements in the *MSH2* and *MLH1* genes seem to be a rare mutational event in Spanish HNPCC patients.

***BRCA1* germ-line mutation status, distinctive cell cycle proteins expression and outcome after breast cancer.** P.O. Chappuis<sup>1</sup>, E. Donato<sup>2</sup>, J.R. Goffin<sup>1</sup>, N. Wong<sup>1</sup>, L.R. Bégin<sup>1</sup>, L. Kapusta<sup>3</sup>, J.-S. Brunet<sup>1</sup>, P. Porter<sup>2</sup>, W.D. Foulkes<sup>1</sup>. 1) Departments of Human Genetics, Oncology, Surgery, Pathology and Medicine; Research Institute of the McGill University Health Centre; Program in Cancer Genetics; Cancer Prevention Centre, Sir M.B. Davis-Jewish General Hospital; McGill University, Montreal, QC H2W 1S6, Canada; 2) Cancer Biology, Fred Hutchinson Cancer Research Centre, Seattle, WA 98109-1024, USA; 3) Department of Pathology, Sunnybrook and Womens College Health Sciences Centre, University of Toronto, Toronto, ONT M4N 3M5, Canada.

**BACKGROUND:** Elevated expression levels of the cell cycle protein cyclin E, and low levels of its inhibitor, p27<sup>Kip1</sup>, have been associated with a poor prognosis following breast cancer. Some studies have found that germ-line mutations in *BRCA1* are also associated with a worse survival. The relationship between cyclin E/p27<sup>Kip1</sup> levels, *BRCA1* status, and outcome has not been studied previously. **METHODS:** We assessed protein levels of cyclin E and p27<sup>Kip1</sup> by immunohistochemistry in an historical cohort of 288 Ashkenazi Jewish women who were diagnosed with breast cancer between 1980 and 1995 and were previously tested for *BRCA1/2* mutations. Breast cancer specific survival (BCSS) was the main outcome measured. **RESULTS:** The median follow-up was 8 years. Thirty tumors carried germ-line *BRCA1* mutations. These tumors were more likely to have high cyclin E expression levels (odds ratio (OR): 9.5,  $P < 0.001$ ) and low p27<sup>Kip1</sup> expression levels (OR: 2.8,  $P = 0.03$ ) than tumors from patients without *BRCA1/2* mutations. On univariate analysis, high cyclin E expression levels (RR: 2.6,  $P < 0.001$ ), low p27<sup>Kip1</sup> expression levels (RR: 2.3,  $P = 0.006$ ) were significant prognostic factors for a poorer BCSS. In the Cox multivariate model, high cyclin E expression (RR: 2.0,  $P = 0.02$ ) remained statistically significant. In the node negative subgroup, high cyclin E and low p27<sup>Kip1</sup> levels were both independent prognostic markers. **CONCLUSION:** High levels of cyclin E and low levels of p27<sup>Kip1</sup> are characteristic of *BRCA1*-related breast cancer. High expression of cyclin E is an independent marker of poor prognosis following breast cancer.

**Analysis of polymorphisms in genes encoding proteins interacting with BRCA1 in high-risk non-BRCA1/2 families.** *F. Guenard*<sup>1</sup>, *Y. Labrie*<sup>1</sup>, *G. Ouellette*<sup>1</sup>, *M. Houde*<sup>1</sup>, *J. Simard*<sup>1,2</sup>, *INHERIT BRCA1*, *F. Durocher*<sup>1</sup>. 1) Cancer Genomics Laboratory and; 2) Canada Research Chair in Oncogenetics, CHUL Research Center, CHUQ, Laval University, Quebec, Canada.

The BRCA1 and BACH1 proteins form a complex known to be involved in double-strand break repair. The BARD1 protein also interacts with this complex. Based on their interaction with BRCA1 and their involvement in other cancers we analysed variations in the sequence of *BACH1* (BRCA1-associated C-terminal helicase 1) and *BARD1* (BRCA1-associated RING domain 1) to verify their possible implication in breast cancer susceptibility in nearly 50 high-risk families for which no mutation in BRCA1/2 was identified. Sequencing of the *BACH1* cDNA led to the identification of two variants: a silent 2637GA change (allele frequency of A:0.40) and a P919S change (frequency S:0.50) located within the exon 19, upstream from the helicase domain. Both polymorphisms also fall into or near the region of BACH1 which binds to BRCA1 (residues 888 to 1063). To examine further other proteins involved in this complex we sequenced the coding region of *BARD1*. BARD1 is associated with the BRCA1 RING domain and the formation of the BRCA1-BARD1 complex is essential for tumor suppression activity. Five different variants were identified in this gene, one of which, 1518CT is a novel conservative variation at codon 506. Two variations (1518CT, V507M) are located in the ankyrin repeats suggesting that it could alter interactions with other proteins and have a frequency of 0.24 and 0.38, respectively. Another variant observed (P24S) is located close to the BRCA1 interacting domain and was found with an allele frequency of S:0.40. The last two variants, a silent change at nucleotide 1053GC and a non-conservative change C557S have an allele frequency of C:0.15 and S:0.02, respectively. As expected both polymorphisms in *BACH1* are in complete linkage disequilibrium (LD). The 5 variants in *BARD1* are also in strong LD and are located more than 57kb apart. Further studies are in progress to establish the profile of polymorphisms in these interacting genes which could have an impact on the stability of the complex when taken together.

**Microarray-based resequencing of the APC gene.** S. Cowie<sup>1,2</sup>, S. Drmanac<sup>3</sup>, D. Swanson<sup>3</sup>, D. du Sart<sup>2</sup>, K. Delgrosso<sup>1</sup>, R. Drmanac<sup>3</sup>, S. Surrey<sup>1</sup>, P. Fortina<sup>1</sup>. 1) Department of Medicine, Thomas Jefferson University, Philadelphia, PA, USA; 2) Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Victoria, Australia; 3) Callida Genomics, Sunnyvale, CA, USA.

Familial Adenomatosis Polyposis (FAP) is an autosomal dominant inherited form of colorectal cancer, caused by mutations in the APC gene. A wide variety of mutations has been found in the APC gene (5q21), the majority of which result in truncated proteins. Several methods of mutation detection are used, but due to the size of the gene, and the large number of mutations, results can take several months. A new quick method of detection which employs an array-based resequencing approach with combinatorial sequencing-by-hybridization (SBH) has been studied.

We initially focused on the "*second hit predictor*" (SHP) region, which contains 167 of 187 known APC mutations from codons 1194-1392 in both FAP and sporadic colon cancer. SHP is centered around codon 1309, which is the most frequently mutated site. We generated approximately 0.5, 2, 4 and 10 kb PCR products encompassing the SHP region and a much more expansive area including the entire exon 15. Each target was subjected to combinatorial SBH. A universal probe set is attached to a solid support and a second one is in solution and labelled with a fluorophore. Ligation occurs only when a complementary DNA strand from the patients PCR product perfectly anneals to both array-bound and solution phase, labelled probe, creating one long labelled probe attached to the surface. A standard array reader scores fluorescent signals at each array position.

Results are very robust for a complete sequence read out for the 0.5, 2 and 4 kb targets. This universal chip successfully detects various base substitutions and insertions, and small base deletions. We are attempting to define the longest DNA stretch (or total combined length of a pool of DNA amplicons) that can be sequenced on a single array in preparation for a continuous read out of the entire APC coding region.

**Mutation spectrum of the BRCA1 and BRCA2 genes in western Sweden.** *A. Bergman<sup>1</sup>, Y. Engwall<sup>1</sup>, J. Lundberg<sup>1</sup>, T. Martinsson<sup>1</sup>, Z. Einbeigi<sup>2</sup>, P. Karlsson<sup>2</sup>, A. Wallgren<sup>2</sup>, J. Wahlström<sup>1</sup>, M. Nordling<sup>1</sup>.* 1) Dpt of Clinical Genetics, Göteborg University, Sweden; 2) Dpt of Oncology, Göteborg University, Sweden.

Germline mutations in the hereditary breast/ovarian cancer causative genes BRCA1 and BRCA2 are considered to constitute approximately 6-10 % of these cancers. The frequency of female mutation carriers with breast/ovarian cancer depends on the population studied, and display considerable variation in coincidence with ethnic and geographical diversity. Mutations are mainly found as small insertions, deletions or substitutions, but also as exon-wide deletions. We performed mutation analyses in 146 patients, selected under informed consent, from the Sahlgrenska University Hospital, Gothenburg, Sweden. Following screening for the western Swedish founder mutation BRCA1 3171ins5 and other Swedish founder mutations, the BRCA1 and BRCA2 genes were screened using the Protein Truncation Test (PTT) for truncating mutations in exon 11 in BRCA1 and exon 10 and 11 in BRCA2. Remainder of the exons was analyzed with either DHPLC or automated DNA sequencing. DNA sequencing of the detected mutations revealed ten different frameshift mutations, four nonsense mutations and one large deletion. Five of these have not been reported earlier: BRCA1 409-410delCA; 2229-2230delAA; 3029delA; 1912 T>G and 3433delA. Mutations in either of the two BRCA genes were detected in 35 % of the screened families; this is comparable to frequencies reported in other European studies. Notably, a western Swedish founder mutation (BRCA1 3171ins5) accounted for 35 of the 51 mutations detected in the 146 families. Results from further investigations made in search for large genomic deletions, where the novel quantitative method multiplex ligation-dependent probe amplification (MLPA) have been used, will be presented at the meeting.

**A sequence variation in exon 1 of the VHL gene associated with reduced penetrance of VHL-disease. A.M.H.**

*Flodin<sup>1</sup>, Y. Engwall<sup>1</sup>, J. Wahlström<sup>1</sup>, L. Wiklund<sup>1,2</sup>, M. Nordling<sup>1</sup>.* 1) Dpt of Clinical Genetics, Sahlgrenska University Hospital, Göteborg, Sweden; 2) Dpt of Neurology, University Hospital, Uppsala, Sweden.

Von Hippel-Lindau (VHL) disease is an autosomal, dominantly inherited tumor syndrome with high morbidity and mortality caused by mutations in the VHL gene. Advances in genetic and clinical management has improved the prognosis for VHL-patients, who develop hemangioblastomas, retinal angiomas, renal cancer and pheochromocytomas. Since 1995 we provide genetic and clinical investigations for Swedish VHL patients. All mutations detected so far by our group are found downstream of codon 65 and they all seem to be highly penetrant. We here report, however, observations in two families concerning codon 38 and 39 in exon 1 of the VHL gene. A 14 year old boy developed a cerebellar hemangioblastoma and was referred to us under the provisional diagnosis VHL. A sequence variation VHL 328G>C (GGC>CGC, Gly39>Arg) was detected in this proband. The variant was also detected in 3 relatives; a child of 11 years and two adults of 49 and 53 years of age. Extensive clinical investigation of these individuals, including ophthalmologic examinations, and scans of brain and upper abdomen, failed to detect any VHL manifestation. Our conclusion is that this sequence variation presents a mutation with reduced penetrance or a polymorphism. These findings seem contradictory to the previous report of a mutation in codon 38 (Li et al, Hum Mut Suppl 1:S31-33,1998). But, this Swedish family was also referred to us, and we failed to detect any sequence variation in that proband or her descendants, suggesting that the previous report was erroneous. The VHL-protein has two known translational initiation sites separated by 53 codons and two proteins with equivalent activity have been detected in cells. A variation in codon 39 should only have an affect on the larger protein suggesting a reduced influence on the activity of the VHL-protein for mutations in the 5-part of exon 1. Sequence variations in this region of the VHL-gene may thus lead to VHL disease with a reduced penetrance.

**BRCA1/BRCA2 Missense Mutations in African-American Breast Cancer Patients.** *L. Baumbach*<sup>1</sup>, *L. Gayol*<sup>1</sup>, *A. Monteiro*<sup>2</sup>, *M.E. Ahearn*<sup>1</sup>, *T. Donenberg*<sup>3</sup>, *A. Gherghina*<sup>1</sup>, *J.F. Arena*<sup>3</sup>. 1) Dept. Pediatrics/Div Genetics, Univ. Miami School of Medicine, Miami, FL; 2) Strang Cancer Prevention Center & Dept. of Cell and Dev. Biol., Weill Medical College of Cornell Univ. New York, NY; 3) FOBCC, Univ. Miami School of Medicine, Miami, FL.

Recent evidence from our laboratory as well as others suggests that specific variants in *BRCA1* and *BRCA2* are associated with increased risk of breast cancer in women of African-American (AA) ancestry. Of particular note is the increasing number of missense mutations/unclassified variants in African-Americans, particularly in *BRCA2*. We are further investigating the association of these variants with disease risk in a cohort of 35 AA probands and their families. The current study is a two-part design: collection of prevalence data for all novel variants, and assessment of functional effects for selected *BRCA1* mutations using a transcriptional assay. *BRCA1* missense mutations were found in 14% (5/35) of the families, while 32% (13/35) of the probands had *BRCA2* missense mutations. Several of the *BRCA1* variants were novel, and we are currently studying their significance. We have determined that *BRCA1* variant, W1718C, which was detected in one family segregating with breast cancer, exhibits a proven loss-of-function phenotype in transcriptional assays. Regarding *BRCA2* missense mutations 5/13 (38%) missense mutations were novel, four of these were found in one family each, with two (E425E, V2820V) being the only variant detected. One variant, S2414S, was detected in two unrelated probands with breast cancer at less than 40 years of age. Prevalence data for 3 of the novel mutations (T777T, E425E, D1923A) are currently being collected. Thus far, for T777T and D1923A, each of these was not present in 80-100 AA control chromosomes. Evaluation of the biological effect of novel *BRCA2* missense mutations awaits further development of a *BRCA2* functional assay. Completion of this study should reveal important information regarding the association of *BRCA1/BRCA2* missense mutations with breast and ovarian cancer risk in AA women.

**BRCA1/2 mutation spectrum and frequencies in Belgian breast/ovarian cancer families.** *K. Claes, B. Poppe, I. Coene, A. De Paepe, L. Messiaen.* Center for Medical Genetics, Ghent Univ Hosp, Gent, Belgium.

World-wide variation in distribution and frequencies of BRCA1/2 mutations is well recognised and for the Belgian population no comprehensive studies have been published. We analyzed the complete coding region of both genes in 300 Belgian breast/ovarian cancer families referred to a family cancer clinic by a combined approach of the protein truncation test (PTT) or direct sequencing (DS) for BRCA1 and BRCA2 exon 11, and heteroduplex analysis (HA) or denaturing gradient gel electrophoresis (DGGE) for screening all other exons and their splice sites. Furthermore, we implemented in our mutation detection strategy MLPA (multiplex ligation-dependent probe amplification; MRC-Holland), a novel method that allows rapid screening for rearrangements in the BRCA1 gene. In total, we identified 44 BRCA1 and 24 BRCA2 mutations. The large majority of the mutations were recurrent, due to founder effects. The most recurrent mutations were BRCA1 IVS5+3A>G and BRCA2 IVS6+1G>A, accounting for more than one-quarter of all mutations detected. The mutation spectrum identified suggests opportunities for the establishment of a cost-effective molecular screening strategy in Belgian patients. MLPA-analysis is not yet completed for all 300 families, but preliminary data did not reveal a major contribution of large intragenic rearrangements in the BRCA1 gene in Belgian patients. A previously reported relation between cancer risk and the location of BRCA1/2 mutations in the coding sequences was not confirmed in the population studied. We found that mutations in the 5' end of the BRCA2 gene were associated with a significantly increased risk for ovarian cancer relative to the rest of the gene. However, associations between mutation position and phenotype are not sufficiently strong to influence genetic counselling and management of individual families. Large variations in cancer risks were observed within families bearing the same mutation, suggesting the involvement of genetic and/or environmental modifiers. For counselling of affected families it may be wiser to take into account the previous history of the family.

**RET proto-oncogene Cys609Tyr mutation in large 4 generation family with medullary thyroid carcinoma and Hirschsprung disease.** *J.F. Atkin<sup>1,2</sup>, E. Couchon<sup>1</sup>, T.W. Prior<sup>3</sup>*. 1) Molecular/Human Genetics, Columbus Childrens Hospital, Columbus, OH; 2) Ohio State University, Department of Pediatrics, Columbus, OH; 3) Ohio State University, Department of Pathology, Columbus, OH.

Germline mutations in the RET proto-oncogene in the cysteine codons 609,611,618, or 620 are known to be associated with MEN2A, Familial medullary thyroid carcinoma (FMTC), and Hirschsprung disease (HSCR). A newborn male with HSCR was evaluated by genetics because his father also had HSCR. The family pedigree revealed multiple family members with FMTC on the paternal grandmother's side. The family was being followed for FMTC by calcitonin levels but none of the family members realized that there could be an association of FMTC with HSCR or that specific genetic testing could determine which family members might need treatment. At least 12 individuals in 4 generations are affected with FMTC without other evidence of MEN2A; our proband and his father are the only ones with HSCR. Testing for RET proto-oncogene was done because of the known association. Exons 10 and 11 of the RET proto-oncogene were sequenced in our proband. At codon 609 in exon 10 a nucleotide substitution was identified, TGC>TAC, which results in the substitution of tyrosine for the normal cysteine. Although this missense mutation has been observed in FMTC and MEN2A, it has not been previously reported in HSCR. Several family members have now been tested or are in the process so that appropriate surveillance, treatment and counseling can be done. This case stresses the importance of careful pedigree analysis, appropriate genetic testing, and thorough genetic counseling. HSCR with FMTC is now known to be associated with this specific missense mutation.

**Hormone replacement therapy, oral contraceptives, and reproductive history in Jewish Israeli breast cancer patients: comparison of BRCA1/2 mutation carriers with non-carriers.** R. Bruchim Bar Sade<sup>1</sup>, A. Chetrit<sup>2</sup>, A.

Figer<sup>3,6</sup>, M. Papa<sup>4,6</sup>, D. Flex<sup>5</sup>, S. Riezel<sup>5,6</sup>, E. Friedman<sup>1,6</sup>. 1) Oncogenetics Unit, Sheba Medical Center, Tel-Hashomer, Israel; 2) The Gertner Institute of epidemiology, Sheba Medical Center, Tel-Hashomer, Israel; 3) Institute of Oncology, Elias Sourasky Medical Center, Tel-Aviv, Israel; 4) Department of Oncological Surgery, Sheba Medical Center, Tel-Hashomer, Israel; 5) Institute of Oncology, rabin Medical Center, Petach Tikvah, Israel; 6) The Sazckler School of Medicine, Tel-Aviv University, Ramat Aviv, Israel.

To assess the role of reproductive factors, use of oral contraceptives (OC) and hormone replacement therapy (HRT) in modifying breast cancer risk among BRCA1 or BRCA2 mutation carriers, Jewish Israeli women with breast cancer unselected for family history of cancer (n=385) were genotyped for the three predominant Jewish mutations in BRCA1 and BRCA2, and data on reproductive factors OC and HRT use, were analyzed by mutational status using logistic regression analyses. Overall, 28/385 (7.3%) of participants [22/247 (8.9%) of the Ashkenazim] were mutation carriers. The majority of mutation carriers were Ashkenazim (n=22; 78.6%) and were diagnosed with breast cancer at or under age 49 years (n=17; 60.7%). Mutation carriers were significantly more likely than non-carriers to ever use OC (39.3% vs. 20.2% ; p=0.053, HRT (35.7% vs. 13.7% ; p=0.007), and have first menarche at or below 12 years of age (71.4% vs. 40.6% ; p=0.03). Multivariate analysis showed that Ashkenazi women diagnosed with breast cancer at or under the age of 40 years, with a positive family history of breast or ovarian cancer who were ever users of HRT were more likely to be mutation carriers, whereas no differences in OC use or other reproductive factors could be noted between carriers and non-carriers. We conclude that Jewish Ashkenazi women featuring these clinical characteristics should be offered genetic counseling and testing. HRT use is seemingly more prevalent among Jewish Ashkenazi mutation carriers.

**Higher Incidence of Rearrangements in Mismatch Repair Genes Compared to Coding Sequence Mutations Observed in a Case Series of HNPCC patients.** *A. Ganguly, M. Houseknecht, H. Messner, L. Godmilow.* Department of Genetics, Genetic Diagnostic Laboratory, University of Pennsylvania, Philadelphia, PA.

The most common form of hereditary colorectal cancer is HNPCC (hereditary nonpolyposis colon cancer). The disease is caused by mutations in 2 major DNA mismatch repair genes - hMSH2 and hMLH1. This report describes the spectrum of mutations observed in a series of 98 consecutive cases submitted for genetic testing of HNPCC. The patient pool included 26 individuals who fulfill Amsterdam criteria (AC+), 25 individuals with Bethesda criteria (BC+), 29 individuals that are unaffected but "at risk" from high risk families and 18 individuals who represent families with clustering of HNPCC related tumors.

**Methods:** Direct sequencing was performed on all the coding exons and exon-intron boundaries of hMSH2 and hMLH1. This was followed by Southern blot analysis for the presence of germline rearrangements in respective genes.

**Results and Conclusions:** Coding sequence mutations were identified in 11/26(42%) AC+ individuals, 3/25 (12%) BC+ individuals and 4/29 (14%) mutations in the "at risk" group. No mutations were detected in the other group and 6 sequence variations of unknown significance were observed. Recently a deletion in hMSH2 gene was identified in ~10% of the North American HNPCC population. We selected a subset of 24 cases of HNPCC with 10 AC+ cases and 7 BC+ cases. The remaining 7 cases were from "at risk" group. We found 1 (4%) case of the common North American founder deletion in an individual with endometrial, colon and renal cancer. In contrast, we found a second common mutation, deletion of 2.3kb between exons 2 and 4 of the hMSH2 gene, 6 times accounting for 25% of the total cases and can be separated as in 2/10 (20%) AC+ individuals, 3/7 (43%) BC+ families and 1/7 (14%) individual without any family history but incidence of sebaceous cancer only. Three other rearrangements were observed within the hMLH1 gene that have not yet been characterized. In conclusion, while coding sequence mutations accounted for 22.5% of the cases in our series, rearrangements within the hMSH2 and hMLH1 genes account for ~50% of the cases.

**A ganglioglioma in a patient with Sotos Syndrome confirmed by a NSD1 deletion.** *M. Deardorff, M. Maisenbacher, E.H. Zackai.* Clinical Genetics Center, Children's Hosp Philadelphia, Philadelphia, PA.

Patients with Sotos syndrome (OMIM 117550), a classical overgrowth syndrome, have been noted with an increased risk of tumor formation estimated to be as high as 2.2% (Cohen MM, Jr. 1999, Am J Med Genet 84:173-5). Types of tumors reported have been varied, but have included Wilms tumors, neuroblastoma and teratomas as well as other isolated tumor types making surveillance strategies difficult. The diagnosis of Sotos Syndrome in these patients has been based on clinical data. Recently, point mutations or deletions of the NSD1 gene (Kurotaki et al., Nat Genet 30:365-6 (2002)) have been reported in patients with Sotos Syndrome. Here we present the first patient with Sotos Syndrome documented to have both a mutation in NSD1 and a tumor, specifically a ganglioglioma. The patient presented at 2 years of age with complex partial seizures. MRI showed a left hippocampal mass. Due to developmental delay and increasing seizure frequency, the mass was resected at 5 years of age and was noted histologically to be a ganglioglioma. He was subsequently seen at 6 years and 9 months of age by clinical genetics due to persistent developmental delay. Symmetric overgrowth, receding hairline, prominent forehead and hypertelorism led to the clinical diagnosis of Sotos syndrome which was confirmed by a microdeletion in the NSD1 locus. Gangliogliomas are relatively rare childhood brain tumors comprising approximately 1% of childhood brain tumors. They have not been observed in other patients with Sotos Syndrome. The diagnosis of Sotos Syndrome in this patient helped to elucidate the cause of the developmental delay which was initially believed to be secondary to the ganglioglioma. This case serves to underscore the benefit of collaboration between clinical genetics and oncology particularly in syndromes known to be associated with tumor formation.

**Further evidence to support that chromosome 15 may be harboring a susceptibility variant in families with multiple polyps.** *D. Daley*<sup>1,5</sup>, *S.D. Markowitz*<sup>1,3,4</sup>, *R.C. Elston*<sup>1,3</sup>, *S. Lewis*<sup>1</sup>, *C. Ticknor*<sup>1</sup>, *P. Platzer*<sup>4</sup>, *J. Lutterbaugh*<sup>4</sup>, *M. McMillen*<sup>1</sup>, *B. Baliner*<sup>3</sup>, *J. Willis*<sup>1</sup>, *G.L. Wiesner*<sup>1,2,3</sup>. 1) Case Western Reserve University, Cleveland, OH; 2) University Hospitals of Cleveland, Cleveland, OH; 3) Ireland Cancer Center, Cleveland, OH; 4) Howard Hughes Medical Institute, Cleveland, OH; 5) University of British Columbia, Vancouver, BC, Canada.

Jaeger et al (AJHG 2003) recently reported a common haplotype shared in a large family of Ashkenazi descent, characterized by an uncommon and specific phenotype of multiple colorectal polyps defined as >3 polyps. They conclude that they have found compelling evidence for a high-penetrance colorectal tumor predisposition gene at 15q-q14 (HMPS/CRAC1), and that it remains to be seen whether HMPS/CRAC1 is also important in other ethnic groups. The Cleveland Colon Neoplasia Sibling Study (CNSS), has recently completed a genome scan in 194 families with genotyping performed by the Center for Inherited Disease Research. We identified 32 families (3 of whom are of Jewish descent) with a multiple polyps phenotype, defined as families in which one individual has >5 polyps. All these families have been screened for familial colorectal cancer, including FAP, HNPCC, and APC including the I1307K variant. In the genome scan results we identified a signal in the region of 15q-q14 using Haseman Elston (H-E) regression analysis ( $p=0.01$ ). However, this finding is not limited to the Jewish ethnic group: in fact our findings for linkage are stronger when the 3 families of Jewish descent are excluded ( $p=0.0037$ ), indicating that in our population this signal is not associated solely with the Jewish ethnic group. The peak is located at ~ 30 cM, between markers D15S165 (20.24 cM) and D15S1012 (36.0 cM). Jaeger et al. report a linked haplotype flanked by markers D15S1031 at 26.0 cM and D15S118 at 31.0 cM, with a peak signal at D15S1007 (29.9 cM). The CNSS linkage signal overlaps this region, adding further validation for a susceptibility gene in this region. Our result provides important new evidence that this locus is associated with a multiple polyp phenotype and that it is not limited to families of Ashkenazi descent.

**Sarcoma in familial multiple lipomatosis: using array-based comparative genomic hybridization to identify regions for linkage.** *J. Goren<sup>1</sup>, K. Rinaldi<sup>1</sup>, J.E. Tomaszewski<sup>3</sup>, A.M. Martin<sup>1,2</sup>, L. Aiello<sup>2</sup>, S. Nussen Lee<sup>2</sup>, B.L. Weber<sup>1</sup>, K.L. Nathanson<sup>1</sup>.* 1) Dept. Medicine, Univ of Pennsylvania Sch Med, Philadelphia, PA; 2) Pennsylvania Hospital, Philadelphia, PA; 3) Dept. Pathology, Univ of Pennsylvania Sch Med, Philadelphia PA.

Familial Multiple Lipomatosis (FML) is a rare autosomal dominant disorder with variable expression. It is characterized by multiple subcutaneous benign lipomas in the regions of the trunk, arm, and thighs with sparing of the head, neck, and shoulders. Sarcomas have been occasionally reported in associated with FML; however the frequency of sarcomas in association with FML appears to be rare given the frequency of FML. We present a patient with FML who developed a retroperitoneal sarcoma with features of both liposarcoma and leiomyosarcoma and renal papillary cancer type 1. In order to determine a possible genetic basis for FML, we performed array based comparative genomic hybridization on the different tumor types to determine if there was a common region of deletion. Several regions of deletion were common to the different tumor types, including ones at 1p24.3 and 9p21. The characterization of these deletions can be used to identify specific loci for targeted linkage studies.

**Diagnostic mutational analysis of the entire genomic sequence of the RECQL4 gene in Rothmund-Thomson Syndrome (RTS) patients.** *B. Chong<sup>1</sup>, M.R. Hegde<sup>1</sup>, D.M. Odenbaugh<sup>1</sup>, A. Gannavarapu<sup>2</sup>, L.L. Wang<sup>2</sup>, S.E. Plon<sup>2</sup>, C.S. Richards<sup>1</sup>.* 1) Diagnostic Sequencing Laboratory, Molecular and Human Genetics; 2) Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030.

Rothmund-Thomson Syndrome (RTS) is an autosomal recessive disorder characterized by presence of skin rash, small stature, sparse hair, skeletal, dental and hair abnormalities and a predisposition to osteosarcoma. Mutations in the RECQL4 gene are found in about two thirds of RTS patients and are associated with cancer risk. We have evaluated the usefulness of denaturing high performance liquid chromatography (dHPLC) as a diagnostic tool for scanning the RECQL4 gene for point mutations, small deletions, and insertions. RECQL4 is a relatively small gene with a size of 6kb and 21 exons. Our assay consists of 16 overlapping fragments to amplify the entire genomic sequence of the RECQL4 gene. All PCR reactions were amplified simultaneously using the same reaction conditions in a 96-well format and then analyzed by dHPLC, using empirically determined optimum temperatures for partial fragment denaturation. Since both homozygous and compound heterozygous mutations have been reported in RTS patients, detection by dHPLC requires mixing of wild-type and patient specimens. The approach was validated by amplifying 20 wild type controls to identify variants using dHPLC and subsequently sequenced to identify the SNPs. Previously studied DNA specimens from RTS patients were analyzed by dHPLC and all mutations were confirmed by sequence analysis. We present our validation studies of dHPLC technology for RECQL4 gene analysis in terms of sensitivity and specificity. The aim of this mutational analysis is to offer a clinical test that will aid in diagnosis of RTS and determination of osteosarcoma risk.

**MSH6 mutation analysis in a clinical series of high-risk hereditary colon cancer families.** *C.H. Buzin<sup>1</sup>, C.Y. Wen-Fong<sup>1</sup>, R. Nedelcu<sup>2</sup>, B. Estaki<sup>1</sup>, J.N. Weitzel<sup>2</sup>, S.S. Sommer<sup>1</sup>*. 1) Dept Molecular Genetics, City of Hope Medical Ctr, Duarte, CA; 2) Dept Clinical Cancer Genetics, City of Hope Medical Ctr, Duarte, CA.

Hereditary nonpolyposis colorectal cancer (HNPCC) is associated with mutations in one of five known mismatch repair (MMR) genes. In the most commonly mutated MMR genes, MLH1 and MSH2, mutations are found in only 50-60% of high-risk hereditary colon cancer families who meet Amsterdam criteria. Recent reports suggest that MSH6 may be responsible for a proportion of HNPCC families, and it is believed to be the third most commonly mutated MMR gene. In addition, MSH6 may be associated with atypical HNPCC phenotypes, in particular gynecologic malignancies. Although high level microsatellite instability (MSI) is associated with MMR defects in HNPCC, MSH6 mutations have also been linked to low level MSI. The objective of the current study is to assess the clinical utility of incorporating MSH6 analysis into clinical evaluation of HNPCC. Patients from hereditary colon cancer families, who previously tested negative for MLH1 and MSH2 mutations, were analyzed for germline MSH6 mutations by sequencing all exons and associated intronic splice junctions in DNA extracted from lymphocytes. Four of 22 patients (18%) had sequence alterations, including two with frameshift mutations and two with missense changes. Both missense alterations (A1154D and L396V) change a wild type amino acid conserved during billions of years of evolution (identical through mouse, *Arabidopsis* and yeast). Microsatellite instability analysis, previously performed on tumors from three of the four patients, was high in the patient with the A1154D alteration. Of the two frameshift mutations, one was associated with an MSI-low tumor and a predominance of gynecologic cancers in the family; the other was in a patient with an MSI-high tumor. Taken in conjunction with findings from other studies, our data suggest that proceeding to MSH6 sequencing in cases that are negative for MLH1 and MSH2 may have clinical utility.

**Identification and characterization of CDKN2A mutations in a group of Brazilian at-risk patients for hereditary melanoma.** *P. Ashton-Prolla*<sup>1,2,5</sup>, *L. Bakos*<sup>3</sup>, *G. Junqueira*<sup>5</sup>, *C. Carvalho*<sup>1</sup>, *R. Giugliani*<sup>1,2</sup>, *D. Hogg*<sup>4</sup>. 1) Serviço de Genética Médica, Hosp Clínicas Porto Alegre Brazil; 2) Departamento de Genética, UFRGS, Brazil; 3) Serviço de Dermatologia, Hosp Clínicas Porto Alegre, Brazil; 4) University of Toronto, Canada; 5) Hospital Mãe de Deus, Porto Alegre, Brazil.

A group of at-risk patients for hereditary melanoma was identified in dermatology and oncology clinics in Southern Brazil and invited to participate in a molecular study. During this first phase, we analyzed the CDKN2A gene, which has an established association with hereditary melanoma. Previous studies in North-American, European, and Australian at-risk patients have identified CDKN2A mutations in 15-25% of families with multiple melanoma or pancreatic cancer diagnoses and in around 15% of patients with multiple primary melanomas. The patients included in our study all had melanoma and additionally, fulfilled at least one of the following criteria: a) diagnosis of more than one primary melanoma; b) family history of melanoma; c) family history of pancreatic cancer; d) personal or family history of dysplastic nevi syndrome. Thirty-six patients from 34 families were identified. Of these, 29 (85%) fulfilled the criteria and 25 effectively participated in the study (25/29; 86%). In the group of 25 patients included in this study (from 24 families) only one deleterious CDKN2A mutation was found (1/24 = 4%). One patient presented a sequence variant of unknown significance (-33G>C) which was localized upstream of the initiation codon, and four patients had the A148T polymorphism, which has no functional significance and occurs in 5% of the general population. These results may be related to a relatively small average number of melanoma/pancreatic cancer cases per family or to the presence of other unidentified genetic abnormalities in this group of patients. The study of additional cases is warranted to better address this question.

**The Hereditary Spectrum of Pancreatic Cancer: The Edmonton Cancer Genetics Clinic Experience.** *M.A. Lilley<sup>1</sup>, D.M. Gilchrist<sup>2</sup>*. 1) Alberta Cancer Genetics Program, Cross Cancer Institute, Edmonton, Alberta, Canada; 2) Cancer Genetics Clinic, University of Alberta, Edmonton, Alberta, Canada.

**Objectives:** It is estimated that, in 2003, there will be 30,700 new cases of pancreatic cancer and 30,000 deaths due to pancreatic cancer in the United States. Pancreatic cancer is known to aggregate in some families and has been associated with a wide variety of cancer syndromes including BRCA2, MEN1, Li-Fraumeni syndrome, Ataxia Telangiectasia, FAP, HNPCC and hereditary pancreatitis. Our objective was to review our experience with pancreatic cancer and the range of associated cancer syndromes.

**Methods:** We reviewed the charts of all patients seen in the Cancer Genetics Clinic at the University of Alberta between 1995 and 2002.

**Results:** Forty families reported a personal or family history of pancreatic cancer in the context of a possible hereditary cancer syndrome. Three additional families reported a history of pancreatitis. Twenty-four (56%) of those families were suspected of having a hereditary breast and ovarian cancer syndrome (BRCA2). A further 7 (16%) were suspected of having HNPCC. Only 3 (7%) were felt to be at risk for a site-specific pancreatic cancer syndrome. Another 3 (7%) were suspicious for hereditary pancreatitis. The remaining family histories were suggestive of Li-Fraumeni syndrome, Von Hippel Lindau syndrome or a non-specific cancer predisposition.

**Conclusions:** With such a wide variety of hereditary cancer syndromes associated with pancreatic cancer, an accurate assessment of the family history is essential to determine the most appropriate cancer screening for at-risk family members and guide any molecular testing that may be offered.

**The Prevalence of breast and colorectal cancer among Ashkenazi Jewish Fanconi anemia and Bloom syndrome carriers.** *I. Kedar-Barnes, H. Baris, G.J. Halpern, N. Magal, M.D. Ludman, M. Shohat.* Dept. of Medical Genetics, Rabin Medical Center, Beilinson Campus Petah Tikva, Israel.

Fanconi anemia and Bloom syndrome are rare autosomal recessive disorders marked by chromosome instability. Bloom syndrome and Fanconi anemia complementation group C (*FACC*) are especially prevalent in the Ashkenazi Jewish (AJ) community. A single predominant mutation for each of these conditions has been reported in Ashkenazi Jews:  $BLM^{ASH}$  in Bloom syndrome and IVS4 in *FACC* with a carrier frequency for  $BLM^{ASH}$  of 1/111 and for IVS4 1/70-1/90. It is well established that both conditions are characterized by susceptibility for developing malignancies. However, a question was raised as to whether carriers also have an increased risk for malignancies. Gruber et al (2002) recently found that AJ with colorectal cancer (CRC) are twice as likely to be carriers of  $BLM^{ASH}$  than AJ controls without CRC. The aim of this study was to estimate the cancer rate among  $BLM^{ASH}$  and *FACC* carriers and their families over 3 previous generations in unselected AJ individuals. We studied 42 carriers of *FACC*, 28 carriers of  $BLM^{ASH}$ , and 43 controls, all of AJ ancestry, in order to estimate the relative risk of CRC, breast cancer and other types of malignancies among carriers and their families. All the subjects filled a questionnaire regarding their personal and family history of cancer for three generations back, including siblings, parents, uncles, aunts and grandparents. Among the families of carriers of *FACC* and  $BLM^{ASH}$ , we found no increased incidence of malignancies over three generations as compared with the families of controls. In a total of 333 1st & 2nd degree relatives of  $BLM^{ASH}$  carriers only 35 cancers were reported: 6 cases of breast cancer and 5 of colon cancer. In a total of 466 *FACC* relatives 46 cancers were reported: 9 breast cancers and 13 colon cancers. Controls consisted of 504 family members with 63 reported cancer cases: 11 breast cancers and 11 colon cancers. For  $BLM^{ASH}$  carriers vs. controls the  $p=0.94$  and for *FACC* carriers the  $p=0.23$ . Among the families of carriers of these genes, we found no increased incidence of cancers in at least three generations compared to the controls.

**Primary aldosteronism in a 14 year old with FAP.** *M. Marvin, C. Bos.* Inherited Colorectal Cancer Program, Spectrum Health, Grand Rapids, MI.

Familial Adenomatous Polyposis (FAP) is an inherited cancer syndrome associated with the development of hundreds to thousands of precancerous colonic polyps. Without colectomy, the development of colorectal cancer is inevitable. While the colonic findings are most striking, a number of extracolonic features are variably present. A number of studies have suggested an associated risk for adrenal masses in FAP. While the prevalence of incidentally detected adrenal masses in the general population is 1-3%, the prevalence of adrenal masses in FAP is estimated to be between 7 and 13%. Previous reports describe the adrenal masses seen in FAP patients as histologically similar to those seen in the general population. They are predominantly nonfunctioning adenomas.

We describe a 14 year old boy who presented for colonic screening secondary to a family history of FAP. He was found to have significant hypertension with a diastolic blood pressure of 115. Further evaluations revealed a potassium level of 2.5, an elevated aldosterone level, biventricular hypertrophy, and an aldosterone secreting adrenal cortical adenoma. Genetic testing revealed a truncating mutation in exon 4 (453delA) of the APC gene.

Few cases of hormone secreting adrenal adenomas in FAP patients have been reported in the literature. This case represents one of the youngest reported cases of primary aldosteronism in FAP and illustrates that adrenal adenomas associated with FAP are not always clinically benign. Blood pressure screening should be part of routine surveillance in FAP.

**Unbiased estimation of breast and ovarian cancer penetrances in BRCA1/2 mutation carriers using genetic-test results.** *F. Marroni*<sup>1,2</sup>, *P. Aretini*<sup>2</sup>, *J.E. Bailey-Wilson*<sup>3</sup>, *G. Bevilacqua*<sup>2</sup>, *G. Parmigiani*<sup>4</sup>, *S. Presciuttini*<sup>1,3</sup>, and the *Italian Consortium for Hereditary Breast and Ovarian Cancer*. 1) Dept Biomedicine, Univ. Pisa, Italy; 2) Dept Oncology, Univ. Pisa, Italy; 3) NHGRI/NIH, Baltimore, MD, USA; 4) Dept Biostatistics, JHU, Baltimore, USA.

Accurate estimates of breast and ovarian cancer penetrance in BRCA1/2 mutation carriers are crucial in genetic counseling. Estimation is difficult because of the low frequency of mutated alleles and of the often-uncertain mechanisms of family ascertainment. We maximized the retrospective likelihood of the genetic model (allele frequencies and cancer penetrances in carriers and non-carriers) given the observed test results using a Markov Chain Monte Carlo approach. Parameter estimates obtained by maximizing the retrospective likelihood remain unbiased even when the ascertainment scheme cannot be modeled. The software BRCAPRO was used as a probability calculation tool. We estimated two different sets of four penetrance functions (breast and ovarian cancer in BRCA1 and BRCA2 carriers), which were called linear (two parameters for each function) and quadratic (three parameters), respectively. Total log-likelihood was similar for the two sets of functions, and corresponding penetrance curves were practically indistinguishable, implying one and the same underlying genetic model. The log-likelihood of the new model was 70 units higher than that of the original model. The table below shows the new penetrance estimates of breast and ovarian cancer at age 50 and 70 in carriers of BRCA1 and BRCA2 mutations, in comparison with the values of the original model (in brackets)

	Breast Cancer Age 50	Breast Cancer Age 70	Ovarian Cancer Age 50	Ovarian Cancer Age 70
BRCA1 Carriers	22% (46%)	46% (69%)	12% (11%)	52% (30%)
BRCA2 Carriers	23% (28%)	49% (67%)	2% (3%)	22% (19%)

**The RET receptor is linked to stress response pathways.** *S.M. Myers, L.M. Mulligan.* Dept Pathology, Queen's University, Kingston, ON, Canada.

The *RET* oncogene encodes a receptor tyrosine kinase involved in cell growth, differentiation and survival. Mutations of *RET* cause the cancer syndrome multiple endocrine neoplasia 2 and are found in sporadic medullary and papillary thyroid carcinomas. As a result of these mutations, RET becomes activated, leading to inappropriate stimulation of downstream signals and altered expression of downstream target genes. The nature of these specific targets is as yet not well understood. We have used cDNA microarray analyses to identify genes differentially expressed in response to RET activation. We have generated cell lines stably expressing RET isoforms (RET9 and RET51) and compared their gene expression profiles to those of RET-ve parental cells. We identified 99 ESTs differentially expressed in the presence of RET. Strikingly, we noticed increased expression of three members of the heat shock protein 70 (HSP70) gene family, *HSPA1A*, *HSPA1B* and *HSPAIL*. Validation by northern and quantitative real time RT-PCR (qRT-PCR) analyses suggested that expression of these genes increased >10-20 fold in the presence of RET. The HSP70s are members of a large group of stress response proteins that may act to promote protein folding and maturation or degradation. To assess the relationship of RET, the stress response and carcinogenesis, we looked for other genes related to stress response with altered expression in the presence of RET. Comparing our data from microarray, northern and qRT-PCR, we showed that multiple genes associated with HSP70's role in protein folding and remodeling, including certain HSP40s, *ST13* and *STIP1* were up regulated coordinately with HSP70 and RET. Conversely, a number of genes associated with an HSP70 role in protein degradation, such as some HSP40s, *STUB1* and *UBQLN2* are down regulated in response to RET. Altered expression of stress response proteins has been noted in a number of tumour types. Our data suggest that RET expression may provide a direct mechanism by which HSP70 proteins, and potentially other stress related proteins are modulated in human tumours. We are currently studying the sequence of events leading to RET activation of the cell survival stress response and carcinogenesis.

**Confirmation that an in-frame deletion of 3 bp in exon 3 of *MLH1* disrupts an exon splicing enhancer (ESE) and is the cause of HNPCC in one Quebec family.** *S. McVety*<sup>1</sup>, *W.D. Foulkes*<sup>1,2</sup>, *G. Chong*<sup>1,2</sup>. 1) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Department of Medicine, SMBD-Jewish General Hospital, McGill University, Montreal, QC, Canada.

Mutations in *MLH1* are an important cause of hereditary non-polyposis colorectal cancer (HNPCC). We recently identified a 3 bp deletion located at the 5' end of exon 3 of *MLH1* that, surprisingly, results in deletion of exon 3 from RNA, as detected by PTT. It has been argued that this in-frame deletion causes HNPCC because of altered binding properties of the protein. In contrast, we postulated that this mutation causes HNPCC because the 3 bp deletion disrupts an exon splicing enhancer (ESE). We based this prediction on the observations that the mutation occurs in a purine-rich region previously identified as representing an ESE in other genes, and that ESEs are often found in exons with splice signals that deviate from the consensus signals, as does the 3' splice signal in exon 3 of *MLH1*.

To test our hypothesis, the 3 bp deletion and several other mutations were created by site directed PCR mutagenesis and tested using an *in vitro* splicing assay. Both mutant and wild type exon 3 sequences were cloned into the Exon-Trapping (Invitrogen) plasmid pSPL3 and transiently expressed in Cos-1 cells. Analysis of the RNA indicates that the 3 bp deletion: del 213-215 AGA, a silent mutation: 216 TC, a missense mutation: 211 GC, and a nonsense mutation: 211 GT all cause varying degrees of exon skipping, confirming the presence of an ESE at the 5' end of exon 3. These mutations are situated in a GAAGAT sequence 3 bp downstream from the start of exon 3.

While ESEs may be up to 8 bp in length and are often purine rich sequences, the involvement of a series of adenines at the start of exon 3 in ESE activity has been excluded because the mutation 213 AC of an A adjacent to the ESE upstream does not affect exon 3 inclusion. The exon 3 ESE is not recognized by available motif-scoring matrices, highlighting the importance of RNA analysis in the detection of ESE-disrupting mutations.

**Identification of susceptibility genes for sporadic breast cancer in a genome-wide association study. S.**

Kammerer<sup>1</sup>, R. Roth<sup>1</sup>, M.R. Nelson<sup>1</sup>, J. Rehbock<sup>2</sup>, F. Ebner<sup>2</sup>, J. Atienza<sup>1</sup>, C. Rosette<sup>1</sup>, M. Denissenko<sup>1</sup>, J. Ekblom<sup>1</sup>, A. Braun<sup>1</sup>. 1) Sequenom Inc., San Diego, CA; 2) I. Frauenklinik, Klinikum Innenstadt, University of Munich, Germany.

In the U.S., over 210,000 individuals will be diagnosed with breast cancer this year, with an expected 40,000 deaths resulting from this disease. There are several well-known factors predisposing individuals to develop breast cancer, such as family history, early menarche, late menopause, alcohol consumption, postmenopausal hormone substitution and obesity. However, very few genetic links to sporadic breast cancer have been identified. We employed a set of 28,000 single nucleotide polymorphisms (SNPs) located in gene regions in an association approach to identify susceptibility genes for sporadic breast cancer. Using DNA pools of 272 cases with breast cancer and 276 age-matched controls of German descent, we identified over 50 candidate susceptibility gene regions. By applying a follow-up strategy including SNP fine-mapping and *in silico* as well as experimental biology, we obtained strong genetic and biological support for several of those novel candidates. One of the genes identified is *DLC-1*, a tumor suppressor gene previously described as a candidate for sporadic breast cancer. The encoded protein is highly homologous to a mouse protein that modulates Rho signaling pathways and was found to be deleted or mutated in several human cancer types. Other compelling novel findings include genes involved in chromosomal segregation / repair and in the AP-2 signaling pathway. For some of those candidates we have demonstrated that suppression of mRNA expression by RNAi leads to a decrease of cell proliferation, indicating that inhibition of those genes might be a promising therapeutic approach in breast cancer. In addition, a gene cluster for adhesion molecules was identified in the breast cancer scan well as in a prostate cancer scan. One of the genes in this region has previously been postulated as a predisposing gene for several cancer types.

**Strategies for Early Detection of Breast Cancer in High Risk Women Using Cytologic and Molecular Studies of Ductal Lavage Fluid.** *B. Haddad, L. Cavalli, L. Shanker, M. Freedman, B. Singh, M. Pennanen, M. Liu, A. Gallagher, J. Rone, R. Dickson, C. Isaacs.* Georgetown University, Lombardi Cancer Center, Washington, DC.

We have established a pilot study in which BRCA1 carriers and true negatives with no clinical evidence of breast abnormalities, either by physical exam or mammogram, undergo ductal lavage (DL). The DL fluid is evaluated by routine cytology and then examined for molecular genetic abnormalities such as LOH. Previous work done by our group has demonstrated LOH of the BRCA1 & FHIT wild type alleles in non-malignant breast tissues surrounding breast tumors, suggesting that these are early genetic change which could be used as markers for early detection. To date we have enrolled 30 patients in our study: 20 BRCA1 mutation carriers and 10 controls. DL was attempted on 28 subjects and was successful in 15. Of those, 8 had adequate material for cytologic analysis and only 1, a BRCA1 mutation carrier, demonstrated focal minimal atypia. The remainder had benign cytologic findings. DNA was extracted from the DL supernatant obtained from all 15 subjects for molecular studies. LOH analysis for the BRCA1 and the FHIT genes was completed on 13 subjects, 9 carriers and 4 controls. In 4 of the 9 mutation carriers we found LOH at the BRCA1 allele, and in 2 of these we also found LOH at the FHIT allele. In 1 of the true negatives LOH at BRCA1 and FHIT loci were also seen. In 1 of the 2 BRCA1 carriers with LOH at the BRCA1 and FHIT loci, minimal cytologic atypia was noted. In the other 3 either few cells were obtained or the DL yielded cytologically benign cells. The patient with minimal atypia had a recent unremarkable mammogram and physical exam. Breast FDG-PET scan showed a focal area of increased uptake in her right breast. This was biopsied and found to have invasive ductal carcinoma. She underwent bilateral mastectomy. In this carrier, LOH at the BRCA1 locus was seen in the DL fluid from both breasts. The DL specimen from her right breast (affected) had insufficient material for cellular analysis and focal minimal atypia was seen in the DL sample from the left breast. Accrual to this study is ongoing.

**Molecular and Phenotypic analyses of 35 Familial Wilms tumor pedigrees.** *S. Hanks*<sup>1</sup>, *EA. Rapley*<sup>1</sup>, *MR. Stratton*<sup>1</sup>, *K. Pritchard-Jones*<sup>2</sup>, *N. Rahman*<sup>1</sup>, *Familial Wilms Tumor Collaboration*. 1) Section of Cancer Genetics, Institute of Cancer Research, Sutton Surrey, United Kingdom; 2) Section of Paediatrics, Institute of Cancer Research, Sutton, Surrey, UK.

Wilms tumor (WT) affects 1 in 10,000 children. In 1-3% of cases, Wilms tumor clusters in families due to a presumed underlying genetic susceptibility. Mutations in the *WT1* gene underlie a minority of familial WT pedigrees. Two further familial WT loci, *FWT1* on 17q21 and *FWT2* on 19q13, have been mapped but have not been identified.

We have ascertained 35 WT pedigrees including a total of 93 WT cases. We have screened all families for *WT1* mutations, identifying only 2 families attributable to *WT1*, both of which showed genitourinary abnormalities in males. We have analysed families informative for linkage at *WT1*, *FWT1* and *FWT2*. Seven of 26 evaluable families are unlinked at all three loci indicating that at least one further familial Wilms tumor gene must exist and suggesting that the contribution of currently unknown loci to Wilms tumor susceptibility may be considerable.

The median age of onset of all familial cases was 3 years, but for the *FWT1*-linked families the median age was significantly older at 5.5 years. 9% of familial cases were bilateral and all the bilateral cases were from separate families. Childhood tumors other than WT occurred in 18% of families. 36% of families had some form of congenital abnormality (cryptorchidism, hemihypertrophy, inguinal hernia, short-stature, overgrowth, renal tract anomalies). The pathology of familial tumors was very variable and <20% had detectable nephrogenic rests.

Our analyses further demonstrate the genetic heterogeneity of familial WT susceptibility and suggest phenotypic associations related to underlying molecular sub-groups may exist. Detailed phenotypic and molecular stratification of WT pedigrees will likely facilitate the identification of the underlying predisposition genes. nazneen@icr.ac.uk.

**Invasive breast cancer following bilateral subcutaneous mastectomy in a *BRCA2* mutation carrier.** *L. Kasprzak*<sup>1</sup>, *F. Tremblay*<sup>2</sup>, *M. Galvez*<sup>1</sup>, *F. Halwani*<sup>3</sup>, *W.D. Foulkes*<sup>1</sup>. 1) Program in Cancer Genetics, Departments of Oncology and Human Genetics, McGill University, Montreal, Canada; 2) Department of Surgery, McGill University, Montreal, Canada; 3) Department of Pathology, McGill University, Montreal, Canada.

We report the occurrence of invasive breast cancer in a *BRCA2* mutation carrier 33 years after bilateral subcutaneous mastectomy. A 49 year old G3P2 presented in 2002 with a benign looking subcutaneous nodule in the left breast. Excisional biopsy confirmed the diagnosis of infiltrating ductal carcinoma, apocrine type with associated ductal carcinoma *in situ*, cribriform type. Estrogen receptor status was strongly positive, progesterone receptor moderately positive and HER-2/neu was negative. Subsequently, she was referred for genetic counseling and found to carry *BRCA2*: 6503delTT, a mutation previously described in the French Canadian population. Family history was significant for breast cancer in proband's father who was diagnosed at the age of 77 and mother diagnosed at 79. A paternal cousin also affected with breast cancer was previously identified as a mutation carrier at another institution. Our patient had undergone bilateral subcutaneous mastectomy with immediate implantation of silicone prostheses at the age of 16 due to extensive fibrocystic breast disease with adenosis. At that time, there were two paternal aunts known to have been affected with breast cancer.

Prophylactic bilateral mastectomy, whether subcutaneous or total, significantly reduces, but does not eliminate, the risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers. The extent of risk reduction achieved by subcutaneous mastectomy is limited given that about 5-10% of the mammary tissue remains *in situ*, but is thought to be of the order of >85%. To our knowledge this is the first case of breast cancer after any type of mastectomy in a *BRCA1/2* mutation carrier and therefore the long-term risk of breast cancer following preventive mastectomy is likely to be very low. This case illustrates the importance of long-term follow up and careful consideration of preventive potential of a less extensive surgical approach in *BRCA1/2* carriers.

**Cancer risk in xeroderma pigmentosum homozygotes and heterozygotes.** *R. Moslehi<sup>1</sup>, J. DiGiovanna<sup>2,3</sup>, A. Goldstein<sup>1</sup>, S. Khan<sup>2</sup>, P. Tucker<sup>1</sup>, K. Kraemer<sup>2</sup>.* 1) Division of Cancer Epidemiology and Genetics, NCI/NIH, Bethesda, MD; 2) Center for Cancer Research, NCI/NIH; 3) Brown Medical School, Providence, RI.

Xeroderma pigmentosum (XP), a rare (about 1 in 10<sup>6</sup>) autosomal recessive disease, is characterized by severe photosensitivity, abnormal pigmentation and increased risk of skin cancer in association with defective DNA repair. Eight XP complementation groups (XPA-XPG and XP variant) have been identified. The frequency of clinically normal appearing XP heterozygotes (about 1 in 300) is much greater than XP homozygotes but their cancer risk is not well studied. Elevated cancer risks in association with heterozygosity for mutations in other recessive disease genes such as ataxia-telangiectasia and Bloom syndrome have been reported. The only study to date on the relatives of XP patients reported a significantly higher frequency of non-melanoma skin cancer among the blood relatives of XP patients in 4 families compared to spouse controls (OR=16, p=0.0001) (Swift and Chase, 1979). This study was performed before the XP genes were identified; the carrier status of the relatives was inferred. For 48 patients ascertained at the NIH over the past several decades, we have assigned a complementation group and for 34 of them, we have identified a causative mutation. Genotype-phenotype observations suggest that the type of skin cancer, frequency of internal tumors and manifestations of neurological abnormalities differ in patients belonging to different complementation groups. We have also collected detailed multi-generation pedigrees on 19 patients. Differences in the frequency of melanoma and non-melanoma skin cancers as well as other cancer sites among obligate carriers of mutations in different XP genes may exist. We are performing a molecular epidemiology study of all consenting relatives in these XP kindreds to confirm all cases of cancer in the family and to determine which relatives are mutation carriers. We plan to compare the risk of cancer in the heterozygote carriers of different XP mutations with that of non-carrier relatives and spouse controls. This study may define the cancer risk of carriers of XP DNA repair genes.

**Molecular diagnosis of Hyperparathyroidism Jaw Tumor Syndrome - germline *HRPT2* mutation in an adolescent with recurrent kidney stones.** *D.J. Marsh<sup>1</sup>, H.J. Stalker<sup>2</sup>, A.E. Nelson<sup>3</sup>, B.G. Robinson<sup>1</sup>, R.T. Zori<sup>2</sup>, V.M. Howell<sup>1</sup>.* 1) Kolling Institute, Royal North Shore Hospital, Sydney, NSW, Australia; 2) Department of Pediatrics, University of Florida, Gainesville, FL, USA; 3) Garvan Institute of Medical Research, NSW, Australia.

A 15 year old male presented to the Urology Clinic with recurrent kidney stones and gross hematuria. Radiological assessment identified a 3.0 cm calcification in the right renal pelvis. Family history revealed that his father also had recurrent kidney stones and had previously undergone surgery to remove a gland in his neck. Eight months later, the patient presented to an otolaryngologist for evaluation of a sebaceous cyst below his left earlobe. A right neck mass was noted and subsequent ultrasound identified a well-delineated cystic mass replacing the entire right lobe of the thyroid. Blood pathology revealed markedly elevated serum calcium (13.8 mg/dL, RI: 8.4-10.2 mg/dL) and parathyroid hormone levels (488 pg/mL, RI: 15 - 65 pg/mL) leading to a diagnosis of primary hyperparathyroidism and hypercalcemia. Radiological examination revealed bone changes consistent with hyperparathyroidism and expansile cystic-like lesions in the right mandible. A single parathyroid gland was removed, and histopathology was consistent with parathyroid adenoma. Germline mutations in a newly identified gene, *HRPT2*, have been found in over 50% of families with Hyperparathyroidism Jaw Tumor Syndrome (HPT-JT). gDNA from this patient was analyzed for a mutation in *HRPT2* by dHPLC and sequence analysis. A novel germline mutation in exon 7, R234X, was identified, predicted to prematurely truncate the *HRPT2* protein termed parafibromin. A molecular diagnosis of HPT-JT has now been made for this patient that has implications for other family members. Our findings highlight the importance of considering hereditary hyperparathyroidism when presented with an adolescent with kidney stones and a family history of recurrent kidney stones.

**Microarray and *HRPT2* mutation analysis in parathyroid tumors.** V. Howell<sup>1,2</sup>, C. Haven<sup>2,3</sup>, B. Robinson<sup>1</sup>, L. Delbridge<sup>4</sup>, J. Philips<sup>4</sup>, G.J. Fleuren<sup>3</sup>, H. Dralle<sup>5</sup>, C. Hoang-Vu<sup>5</sup>, O. Gimm<sup>5</sup>, A. Nelson<sup>1</sup>, H. Morreau<sup>3</sup>, D. Marsh<sup>1</sup>, B. Teh<sup>2</sup>. 1) Kolling Institute of Medical Research, Royal North Shore Hospital (RNSH) and University of Sydney, NSW, Australia; 2) Van Andel Research Institute (VARI), MI, USA; 3) Leiden University Medical Center, Netherlands; 4) RNSH and University of Sydney; 5) Martin Luther University, Germany.

The molecular events underlying the formation of parathyroid tumors are poorly understood. Microarray analysis was undertaken to identify unique genetic profiles for these tumors. Fifty-three parathyroid tumors consisting of 38 sporadic (16 adenomas, 16 hyperplasias, 5 carcinomas, 1 lithium associated), & 15 familial (5 MEN 1, 1 MEN 2A, 5 HPT-JT, 4 FIHP) & a pool of 16 normal parathyroid tissues were studied. Microarray analysis of tumor and Universal Human Reference RNA (Stratagene) was performed using slides spotted with 19,968 cDNA clones (Research Genetics Human Clone Set). The normalized results were manipulated in CLUSTER. A subset of tumors was also characterized at the *HRPT2* locus by sequencing & LOH. Unsupervised clustering of the array data revealed 3 broad clusters. Group 1 contained all sporadic carcinomas, all HPT-JT & some FIHP tumors. Group 2 contained the majority of sporadic adenomas, all MEN 1 & some FIHP tumors. Group 3 contained the majority of hyperplasias, the lithium-associated, MEN 2A & the pooled normal specimens. All tumors sequenced in group 1 harbored at least 1 *HRPT2* mutation. Two-hits affecting *HRPT2*, were found in 6 of these 11 tumors. No mutations were found in any of the other specimens sequenced. In conclusion, gene expression profiling has identified distinct genetic groupings of parathyroid tumors. A strong association between intragenic mutations of *HRPT2* & the cluster group containing all sporadic malignant & familial HPT-JT tumors has also been identified & is supportive of a role of *HRPT2* as a tumor suppressor gene in these tumor groups.

We thank the VARI Microarray Core Facility for the microarrays.

VH & CH contributed equally to this work.

**CHEK2 and breast cancer: a validation study in Finland and expression in unselected breast tumors by tissue microarray analysis.** *O. Kilpivaara<sup>1</sup>, J. Bartkova<sup>2</sup>, H. Eerola<sup>1,3</sup>, K. v. Smitten<sup>4</sup>, K. Aittomki<sup>5</sup>, C. Blomqvist<sup>3</sup>, G. Sauter<sup>6</sup>, O.-P. Kallioniemi<sup>7</sup>, J. Bartek<sup>2</sup>, H. Nevanlinna<sup>1</sup>.* 1) Dept. Obs. & Gyn., Helsinki University Central Hospital (HUCH), Finland; 2) Inst. Cancer Biology, Danish Cancer Society, Copenhagen, Denmark; 3) Dept. Oncology, HUCH, Finland; 4) Dept. Surgery, HUCH, Finland; 5) Dept. Clinical Genetics, University of Helsinki, Finland; 6) Dept. Pathology, University of Basel, Switzerland; 7) VTT Biotechnology, Turku, Finland.

CHEK2 is a serine/threonine kinase that has a pivotal role in the cell cycle checkpoint signaling network, contributing to damage-induced transcription, DNA repair, cell cycle arrest, and apoptosis. We (Vahteristo et al., AJHG 2002) and others (The CHEK2-Breast Cancer Consortium, Nat Genet 2002) have previously reported the association of a germ line truncating mutation 1100delC with familial breast cancer; the variant is also found in 1.4% of the normal Finnish population. We have here further analyzed the germ line CHEK2 1100delC in 295 familial breast cancer cases, 285 unselected cases and 30 cases with bilateral breast cancer. CHEK2 1100delC was strongly associated with bilateral breast cancer (3/30, 10.0%,  $p=0.0001$  and with familial breast cancer (21/295, 7.1%,  $p<0.00001$ ), as compared to population controls. These results confirm our previous findings on Finnish breast cancer patients. We also studied the CHEK2 protein expression in breast cancer by immunohistochemical analysis of a tumor microarray of 611 unselected breast tumors. The CHEK2 protein expression was reduced in 21.1% of the breast tumors (93/440 tumors with an IHC staining result). The tumors with reduced CHEK2 expression had more often larger primary tumor size ( $p=0.0017$ ), particularly pT4 class ( $p=0.0003$ ) than those with normal staining. In contrast, no association was seen with hormone receptor or p53 status, histology, grade, lymph node status, or overall survival. In conclusion, these results imply a significant role for the inactivation of the CHEK2 signaling in the genetic predisposition to breast cancer and possibly also in mechanisms of sporadic tumor progression.

**Noninvasive imaging of pancreatic neuroendocrine tumors in MEN1-knockout mice.** A.L. Kennedy<sup>1</sup>, P.C. Scacheri<sup>1</sup>, J. Munasinghe<sup>2</sup>, D. Schimel<sup>2</sup>, J.S. Crabtree<sup>1</sup>, F.S. Collins<sup>1</sup>. 1) NHGRI, NIH, Bethesda, MD; 2) NINDS, NIH.

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant cancer syndrome, characterized primarily by multiple tumors of the pancreatic islet cells, parathyroid glands, and anterior pituitary. Nearly all patients who inherit an inactive copy of the MEN1 gene develop at least one endocrine tumor by age 50. The pancreatic endocrine tumors associated with MEN1 grow slowly and are difficult to recognize and treat. Developing high-resolution imaging modalities for pancreatic endocrine tumors is clearly needed to monitor tumor progression for patients with MEN1. We recently developed a mouse model of MEN1 by disruption of the mouse *Men1* homolog. Heterozygous *Men1* knockout mice develop a tumor spectrum that is remarkably similar to that of the human condition. Currently, necropsy is the only way to detect the tumors in the *Men1* mice, making it quite difficult to evaluate possible therapeutic interventions. The goal of this study was to develop noninvasive methods for detecting pancreatic endocrine tumors in live *Men1* knockout mice. Using Magnetic Resonance Imaging (MRI) we can detect small (1-4 mm) pancreatic neuroendocrine tumors on respiratory-gated images obtained with a multi-slice T2 weighted spin echo sequence. These tumors are distinguishable from the surrounding pancreas and enhanced upon administration of manganese-chloride based contrast agents. Two small (2-3 and 3-4 mm) pancreatic endocrine tumors were also detectable in a separate mouse using micro-CT (Computed Tomography). For micro-CT, the mouse was administered Gastroview to distinguish the stomach and digestive tract from the surrounding visceral organs. The size and location of the tumors detected with MRI and CT were confirmed upon autopsy. Although imaging of a variety of different tumor types in mice has been extensively reported, to our knowledge this is the first report of successful imaging of pancreatic endocrine tumors in live mice. The imaging protocols developed in this study may be adaptable to human patients with MEN1, and will be useful for future studies aimed at inducing tumor regression.

**Variants in Androgen Metabolism Pathway Genes and Testicular Cancer Susceptibility.** *K.L. Nathanson<sup>1</sup>, R. Letrero<sup>1</sup>, K. Robertson<sup>1</sup>, E. Ellis<sup>1</sup>, D.J. Vaughn<sup>1</sup>, P.A. Kanetsky<sup>2</sup>*. 1) Dept Medicine, Univ Pennsylvania Sch Med, Phila, PA; 2) Dept Epidem and Biostat, Univ Pennsylvania Sch Med, Phila, PA.

Testicular cancer is the most common cancer in men ages 20-40. Genetic and environmental factors are postulated to play important roles in testicular cancer development. We examined variants in genes involved in the androgen metabolism pathway including cytochromes P450 (*cyp*)-*3A4*, *3A5*, 5- reductase type 2 (*SRD5A2*) and the androgen receptor (*AR*) among 100 testicular cancer cases and 100 controls frequency matched by age and race. Cases were mostly white (95%). Variants in *cyp3A4*, *cyp3A5*, and *SRD5A2* did not differ significantly between the cases and controls. We hypothesized that repeat sizes in *AR* would be longer in cases than in controls, as testicular cancer is associated with a relatively estrogenic environment. Non-parametric comparisons indicated that cases had significantly longer GGC repeats ( $p=.034$ ), but shorter CAG ( $p=.0024$ ) than controls. We found that longer (17) GGC repeats and shorter (23) CAG repeats were associated with testicular cancer (OR 2.8, 95% CI 1.3-6.6 and OR 2.6, 95% CI 1.25-5.5, respectively). Since *AR* is on the X chromosome, we determined haplotypes based on the above dichotomies. Although not statistically significant, results from the haplotype analysis confirmed the single locus analyses. Compared to carriers of short CAG and short GGC repeats, carriers of short CAG and long GGC repeats were more likely (OR=2.6, 95% CI .97, 6.7), carriers of long CAG and short GGC repeats were less likely (OR=.58, 95% CI .19, 1.8), but carriers of long CAG and long GGC repeats were as likely (OR=1.1, 95% .23, 5.3) to be cases. Controversy exists about the relative importance of the CAG and GGC repeats in modulating androgen responsiveness and several studies in prostate and breast cancers have hypothesized that the GGC repeat is in fact causative with the CAG repeat associations due to linkage disequilibrium. Consistent with prior studies in other cancers, we suggest that longer GGC repeats are associated with decreased androgen responsiveness and thus testicular cancer susceptibility.

**A novel homozygous mutation in the MSH6 gene in a Turcot Syndrome family characterized by pediatric glioblastoma multiforme, lymphoma, colorectal cancer and neurofibromatosis.** *M.R. Hegde<sup>1</sup>, M.A. Blazo<sup>1</sup>, L.H. Chin<sup>1</sup>, P.A. Ward<sup>1</sup>, M.M. Chintagumpala<sup>2</sup>, J.Y. Kim<sup>2</sup>, S.E. Plon<sup>2</sup>, C.S. Richards<sup>1</sup>.* 1) Diagnostic Sequencing Lab, Department of Molecular and Human Genetics; 2) Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030.

Heterozygous mutations in one of the DNA mismatch repair genes (MMR) cause hereditary nonpolyposis colorectal cancer (HNPCC [MIM114500]). Turcot Syndrome [MIM276300] is the association of brain tumors and colorectal cancer (CRC) and has been reported to be both a dominant and recessive disorder. Homozygous and compound heterozygous mutations in MLH1 and MSH2 genes have been reported in five families characterized by hematologic malignancy, CNS tumors, and the neurofibromatosis type I (NF1) phenotype. Here we describe a non-consanguineous Pakistani family including a son who died of lymphoma and colorectal cancer diagnosed at ages five and eight, respectively. His eight-year-old sister presented with glioblastoma multiforme, atypical café-au-lait spots and axillary freckling. There was no history of malignancy in the parents generation and only one distant relative was reported to have CRC. Mutational analysis was performed for MLH1, MSH2 and MSH6 using dHPLC and sequencing to identify the mutation in the sister. A homozygous single base insertion mutation was identified (3634insT) which results in a premature stop at codon 1223 in exon 7 of the MSH6 gene. This mutation is expected to produce a truncated protein. Both parents were found to be heterozygous for the 3634insT mutation. We report here the first identification of homozygous inactivation of the MMR gene MSH6 in a family with pediatric onset brain tumor, lymphoma, CRC and NF1 phenotype. Our findings support a role for MSH6 in Turcot Syndrome and are consistent with a recessive mode of inheritance.

**Transcriptional response of human lymphoblastoid cells to ionizing radiation.** *K.Y. Jen, V.G. Cheung.* Dept Pediatrics, Univ Pennsylvania, Philadelphia, PA.

Humans are exposed to ionizing radiation (IR) through the environment and in medical diagnostic and treatment procedures. The major consequence of IR exposure is the generation of single and double-stranded breaks in DNA. This DNA damage can accumulate resulting in malignant transformation of somatic cells or heritable mutations in germ cells. To counteract this threat, human cells have complex networks of genes and proteins that are involved in response to IR exposure.

In this study, we used oligonucleotide microarrays to investigate the temporal transcriptional response of lymphoblastoid cells to IR. We irradiated lymphoblastoid cells from 10 unrelated individuals at 3 Gy and 10 Gy doses. Expression profiles of the cells prior to radiation treatment and at 1 hr, 2 hr, 6 hr, 12 hr and 24 hr after IR exposure were obtained. We identified 319 and 816 IR-responsive genes following the 3 Gy and 10 Gy of IR exposures, respectively. Among these genes, 126 genes are in common between the two doses. Many of the IR-responsive genes are involved in cell cycle, cell death, DNA repair, DNA metabolism, and RNA processing.

We will present data from our study and discuss the temporal expression profiles of the IR-responsive genes and dose-dependent IR-response in normal human cells. We will also compare the results to the gene expression profile of cells from patients with radiosensitive diseases. These findings are important for understanding processes such as radiation-induced carcinogenesis, radiation sensitivity, and the development of biomarkers for radiation exposure.

**Characterization of the *Drosophila fancd2* Homologue.** *K. McCabe*<sup>1</sup>, *C. Clay*<sup>2</sup>, *M. Forte*<sup>2</sup>, *M. Grompe*<sup>1</sup>. 1) Dept Medical & Molec Genetics, OHSU, Portland, OR; 2) Vollum Institute, OHSU, Portland, OR.

Fanconi anemia (FA) is an autosomal recessive cancer susceptibility syndrome characterized by birth defects, progressive bone marrow failure, and cellular hypersensitivity to DNA crosslinking agents. Six genes have been cloned (*FANCA*, *C*, *D2*, *E*, *F*, and *G*) and bi-allelic inactivation of *BRCA2* was shown in FA-B and D1 patients, genetically linking the FA and BRCA pathways. FANCD2 monoubiquitination in a cell cycle and DNA damage dependent manner requiring the FA complex (*FANCA*, *C*, *E*, *F*, and *G*), and FANCD2 phosphorylation following ionizing radiation by ATM indicate FANCD2 may be the branch point between DNA inter-strand crosslink and double-strand break repair. Perhaps most intriguing, *FANCD2* is the only FA/BRCA gene in the pathway to have a homologue outside of vertebrate organisms and may represent the evolutionary precursor of the FA/BRCA global genome repair pathway. A recently constructed 130Kd HA tagged *fancd2* will now permit us to determine if *fancd2* is post-translationally modified in a manner consistent with mammalian FANCD2. Preliminary results from RNAi knock down experiments of fly *fancd2* in Kc cells indicate there is no increase in IR sensitivity at the cell level, consistent with observations in mammalian systems. RNAi experiments examining inter-strand crosslinker sensitivity are underway. In addition, we have developed a transgenic fly carrying a *GAL4* inducible *fancd2* RNAi construct. By breeding this fly to lines carrying *GAL4* driven by various promoters we can knock-down *fancd2* in a spatially and developmentally controlled manner. Also under development is a *fancd2* knock-out fly. The results from these experiments will demonstrate if fly *fancd2* is indeed an orthologue of the FANCD2 of vertebrates. If the *Drosophila fancd2* homologue is the progenitor of the FA/BRCA pathway, future experiments examining fly *fancd2* may provide insight into the function of FANCD2 in mammals. Utilizing *Drosophila fancd2* as a model for FA/BRCA global genome repair will provide a biochemical foothold on the mammalian pathway, permitting acceleration of our understanding of Fanconi anemia, cancer predisposition syndromes, and DNA repair.

**Using synthetic lethality in *Drosophila melanogaster* for drug target discovery.** L.R. Marek, V. Busygina, A.E. Bale.  
Dept Genetics, Yale Univ, New Haven, CT.

Synthetic lethal screens are used in yeast to identify novel mutations which are not themselves lethal, but cause lethality in combination with mutations in a gene of interest. These screens have the potential to identify genes that interact with known cancer genes; thereby, identifying a novel anticancer drug target. However, many cancer genes are not conserved between yeast and humans. *Drosophila*, on the other hand, bear homologs to nearly every key cancer-related gene. For this reason, our laboratory performed a synthetic lethal screen using flies to identify novel drug targets for tumors associated with Multiple Endocrine Neoplasia Type I (MEN1).

MEN1 is an autosomal dominant disease characterized by tumors of the endocrine glands. Tumors arise from loss of both copies of the gene, MEN1. Because homozygous loss of MEN1 in *Drosophila* results in viable flies, a synthetic lethal screen was performed using MEN1 mutant flies as a model for MEN1 tumors. Flies that were homozygous mutant for MEN1 were tested against 83 deletion lines that covered chromosome 3. A single deletion line was identified that causes lethality in MEN1 mutant flies. Specifically, in a cross that generated 167 flies in the F1 generation, 25% of the progeny was expected to be MEN1 mutants carrying the deletion. Zero flies hatched with this genotype, indicating that a gene located in this deletion is interacting with MEN1 to cause lethality. This deletion spans approximately 2200 kb, deleting 248 genes. Using a series of 9 smaller deletion lines, 95 genes have been eliminated and the search will eventually be narrowed to 10-15 candidates. Genetic crosses with known mutants in the region and RNAi will ultimately identify the gene responsible for lethality. This screen has the potential to determine new drug targets whose inactivation in combination with the loss of MEN1 kills endocrine tumor cells specifically. This study is the first to validate *Drosophila* as a model organism for synthetic lethality drug target screening, and sets the stage for large-scale studies to systematically screen every gene in the *Drosophila* genome.

**Is there a biological basis for A-T heterozygote cancer risk.** *M. Lavin.* Dept Cancer Biol, QIMR, Brisbane, Australia.

The human genetic disorder ataxia-telangiectasia (A-T) is characterized by neurodegeneration, immunodeficiency, radiosensitivity, cell cycle defects, genomic instability and cancer predisposition. A-T is an autosomal recessive disorder but there is evidence for penetration of some aspects of the phenotype in heterozygotes. Carriers of a defective ATM (ataxia-telangiectasia mutated) gene exhibit intermediate levels of radiation-induced chromosome aberrations and cells in culture are intermediate between A-T and controls in their sensitivity to ionizing radiation. In addition several epidemiological studies have pointed to an increased risk for cancer, especially breast cancer, in A-T heterozygotes. However, initial mutation analysis on the ATM gene in patients with breast cancer did not correlate with the epidemiological data. It seems likely that this was due to the nature of the mutations screened for. More recent data suggest that the presence of missense mutations in ATM lead to a dominant interfering effect on wild-type ATM protein thus compromising its activity. This mechanism of interference on normal ATM function and cancer predisposition in A-T heterozygotes is supported by studies with animal models. The biological basis for increased cancer incidence in carriers of the ATM defective gene will be discussed.

**MEN1 testing in a clinical diagnostic laboratory.** *R.D. Klein, J. Bessoni, A.E. Bale.* Yale University School of Medicine, New Haven, CT.

MEN1 is an autosomal dominant syndrome characterized by parathyroid, pancreatic islet, pituitary, and occasionally, other tumors. Previous functional studies of the MEN1 protein, "menin", suggest a role as a transcriptional co-modulator. The MEN1 gene has an 1830 bp coding region spanning exons 2 through 10. Disease-related mutations of the gene occur in all portions of the coding sequence and most often produce truncated proteins. Missense mutations also occur, but there is little evidence for genotype-phenotype correlations. From October 1997 - May 2003, blood samples from 398 individuals of 287 pedigrees were received for clinical MEN1 testing. In each proband, exons 2 - 10 with the intron-exon junctions were PCR amplified and sequenced (ABI 377). Among 94 (presumably unrelated) pedigrees that tested positive, 72 mutations were found. The mutational spectrum included 17 nonsense mutations (23.6%), 18 frameshift deletion mutations (25%), 10 frameshift insertion or duplication mutations (13.9%), 11 splice site mutations (15.3%), 2 in-frame deletions (2.8%), and 14 probable missense mutations (19.4%). In-frame deletions and missense mutations were judged to be deleterious if they were not found in 200 unaffected individuals, altered highly conserved bases, and segregated with the disease in families. Among the recurrent mutations, 4 were found in >2 pedigrees including a 4-base deletion in codons 83-84 that was present in 6% of the affected pedigrees. Recurrent mutations in apparently unrelated pedigrees suggest the presence of founder effects or mutational hotspots. Broad haplotyping of the MEN1 region to distinguish these possibilities is in progress. Individuals most likely to test positive were those from families affected with parathyroid and pancreatic islet neoplasia +/- other features. Sporadic patients with these tumors were somewhat less likely to test positive, possibly reflecting somatic mosaicism. A substantial proportion of families with hyperparathyroidism alone tested positive, but the combination of parathyroid and pituitary disease without pancreatic islet neoplasia was rarely associated with an MEN1 mutation. This latter combination of tumors may represent a different genetic disease.

**Increased identification of patients at risk for hereditary colorectal cancer using a questionnaire.** *T. Monteith<sup>1</sup>, R. Bostick<sup>2</sup>, K. Brooks<sup>1</sup>, M. Seabrook<sup>3</sup>, S.R. Young<sup>1</sup>.* 1) Dept. of Ob/Gyn, USC, Columbia, SC; 2) Dept. of Family Practice, USC, Columbia, SC; 3) Consultants in Gastroenterology, Columbia, SC.

Every year, 150,00 new cases of colorectal cancer (CRC) are identified in the U.S. Of these, 5-10% are thought to be inherited. Hereditary non-polyposis colorectal cancer (HNPCC) is responsible for approximately 3-5% of CRC. Unlike familial adenomatous polyposis, HNPCC is similar in clinical presentation to sporadic CRC. A common belief is that a substantial proportion of persons at risk for HNPCC are not identified. This study's goal was to evaluate a simple, self-administered screening questionnaire to identify patients at risk for hereditary CRC, particularly HNPCC. Potential participants were sequential patients attending outpatient pre-colonoscopy appointments at a large endoscopy center. A total of 1005 patients (100% consent rate) completed the questionnaire, and were classified into four groups based on family history: 1) negative (414), 2) isolated cancer (210), 3) more than one cancer but not meeting Amsterdam criteria or Bethesda guidelines (258), and 4) meeting Amsterdam criteria or Bethesda guidelines (123). Chart reviews were conducted on group 4 as well as a randomly selected equal number from the remaining three groups. Of participants on whom chart reviews were conducted, 51 (10%) were randomly selected for family history interviews. Telephone interview was a priori considered to be the criterion standard method for identifying at risk families. The three methods (usual care, questionnaire, and interview) were compared using the Kappa (K) statistic. The questionnaire and interview were in substantial agreement (K = .672), but the chart and questionnaire and chart and interview were in moderate agreement (K = .456 and .473, respectively). These findings suggest that the use of a screening questionnaire can lead to a substantial increase in the number of patients identified as being at increased risk for hereditary CRC, especially HNPCC. Findings also suggest that a major reason that risk for HNPCC may be overlooked in usual gastroenterology clinical care is not soliciting or considering family histories of extra-colonic HNPCC-associated cancers.

**Mutational Analysis of the Mismatch Repair Genes in HNPCC Patients.** *K. Nafa, P. Peterlongo, J. Shia, L. Canale, G. Lerman, E. Glogowski, J. Guillem, A. Markowitz, K. Offit, N.A. Ellis.* Memorial Sloan-Kettering Cancer Center, New York, N.Y.

Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant cancer predisposition syndrome predominantly caused by germline mutations of the MLH1, MSH2, and MSH6 mismatch repair (MMR) genes. We have ascertained 126 families, most of which had at least three first or second degree relatives diagnosed with colorectal cancer at any age (HNPCC-like), to obtain a clinical cohort that will be useful in gene discovery. 64 families met Amsterdam criteria (AC) I or II and 54 families met HNPCC-like criteria; 8 families met Bethesda criteria. By microsatellite instability (MSI) analysis, 38 of the 95 families tested exhibited MSI (MSI-high). We used DHPLC to identify mutations in MLH1, MSH2, and MSH6 in an affected person from each of the 38 MSI-high families (31 AC, 4 HNPCC-like, 3 Bethesda) and the 31 MSI-untyped families (19 AC, 9 HNPCC-like, 3 Bethesda). Deletion analysis was performed by semi-quantitative fluorescent multiplex PCR in families lacking mutations by DHPLC analysis. Here we report 34 unique mutations identified in 47 persons (39 AC, 5 HNPCC-like, 3 Bethesda)--15 mutations in MLH1, 17 in MSH2, and 2 in MSH6. The mutation types consisted of 9 frameshift mutations, 1 nonsense mutation, 11 splice site mutations, 1 deletion of exon 8 of MSH2; 10 amino acid substitutions, and 2 single amino acid deletions. The MSH2\*IVS5+3A>T mutation was detected 5 times, the MLH1\*1411delAAGA and MLH1\*1852delAAG mutations were detected 2 times each, and the MSH2\*1906G>C (A636P) mutation was observed 8 times, occurring only in Ashkenazi Jewish families. Mutations were identified in 31 of the 38 (0.82) MSI-high families and 16 of the 31 (0.52) MSI-untyped families even though family history was similar in both groups, suggesting that MSI screening increases the mutation detection rate. In the 7 MSI-high mutation-negative families, undetected mutations may be present. With 72 (20 AC, 48 HNPCC-like, 4 Bethesda) families putatively lacking a MMR gene mutation, the cohort assembled is a starting point for the identification of previously unknown genetic susceptibility factors.

**Changes of gene expression in leukemia cells surviving ultra-short pulsed electric fields.** *M. Stacey<sup>1</sup>, P. Fox<sup>1</sup>, D. Trang<sup>1</sup>, K. Schoenbach<sup>2</sup>, S. Beebe<sup>1</sup>.* 1) Ctr Pediatric Research, Eastern Virginia Medical Sch, Norfolk, VA; 2) Ctr Bioelectrics, Old Dominion Uni, Norfolk, VA.

Ultra-short, high intensity, pulsed electric field (nsPEF) applications, and the ability to expose cells to them, have emerged from recent advances in electronics. nsPEF exposures are of high electrical intensity with pulse duration in the order of nanoseconds (billionths of a second). These are conditions that cells are unlikely to have encountered during evolution and it is therefore largely unknown how cells react to such exposures. We have measured the survival of different cell lines exposed to nsPEF and found that cells derived from leukemia and from transformed lymphoblastoid B-cells derived from patients with cancer predisposing syndromes are specifically sensitive to the killing effects of nsPEF (approx 10% survival). Those derived from solid tumors do not show the same sensitivity (70-100% survival). Surviving leukemia cells showed a significant reduction in the number of cells reaching mitosis (%mitotic index;%MI) for 4 days post-exposure compared to unexposed cells, before returning to normal levels after one week. Cell lines derived from solid tumors did not show a reduction in %MI at any time point. Here we present findings of microarray analysis on surviving cells describing changes in gene expression associated with cell survival and discuss the use of antisense drugs to maximize cell killing in leukemia cells.

**Family history of breast cancer in Mexican men with breast cancer.** *S. Vidal, M. Chavez, A. Fuentes, D. Cantu.*  
Dept Clinical Research, Inst Nacional de Cancerologia, Mexico City, Mexico.

Male breast cancer is a rare entity and accounts for approximately 1% of all breast cancers. Consequently, much less information is available regarding the disease in men. Geographic and temporal variation in the rates is modest suggesting limited environmental and life-style influence. Information regarding male breast carcinoma is nearly entirely retrospective and uncontrolled. Although the etiology of breast cancer in men is not understood, hormonal and genetic factors both appear to play a role. An increased risk of breast cancer in men has been associated with a family history of breast cancer. We retrospectively reviewed charts of all breast cancer patients between 1980 and 2001 diagnosed and treated at the National Cancer Institute in Mexico to evaluate the family history of breast cancer in these patients. In this period 10,671 breast cancers were treated in our institution, of these cases 23 were male, representing 0.21% of all cases. All of them were Mexican, age ranged from 25 to 81 years (mean 56.5). Only 2 patients (8.6%) had family history of breast cancer, 1 of them has a maternal aunt with unilateral breast cancer and the other one with bilateral breast cancer has 2 first degree relatives and 5 second degree relatives affected with breast cancer, all of them women from maternal side. Even previous investigators have indicated that family history is one of the strongest risk factors for breast cancer in men and women in this study just 2 cases were found. Clearly one of them has breast and ovarian cancer syndrome and the rest of the family must be evaluated for BRCA2 mutation. These results suggest that in Mexican patients the frequency of familial breast history is not so common. However, we can not reject the possibility that the information given for the patients were incomplete due that much of our population comes from little towns without medical services and they ignore the real cause of death of their relatives.

***PTEN* Mutation in an Adult Male Fulfilling Clinical Criteria for Bannayan-Riley-Ruvalcaba-Smith and Cowden Syndromes.** *N. Wong*<sup>1</sup>, *J.R. Jass*<sup>2</sup>, *W.D. Foulkes*<sup>1,3</sup>. 1) Cancer Prevention Centre, SMBD-Jewish General Hospital, McGill University; 2) Dept. of Pathology, McGill University; 3) Program in Cancer Genetics, McGill University.

Germline mutations in *PTEN* are known causes of Cowden syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome (BRRS), two hamartoma syndromes which clinically overlap. CS is characterized by multiple hamartomas of the skin, breast, thyroid, oral mucosa, GI tract, and CNS. Hallmarks features of CS are trichilemmomas and mucocutaneous papules seen in >90% of patients. Breast and thyroid cancer are also part of CS. About 40% of CS patients are macrocephalic. Typical features of BRRS include macrocephaly, lipomatosis, intestinal hamartomatous polyps and speckled penis. Increased risk for malignancy is thought not to occur in pure BRRS families, whereas in CS, lipomatosis is rare and pigmentation of the glans penis has never been reported. We report an individual with a novel mutation, Y16X, in *PTEN* meeting the clinical criteria for both CS and BRRS.

The proband is an A. Jewish male referred for colonic polyps. Physical exam revealed a head circumference >97 percentile, a prominent forehead, oral papules, florid axillary papillomas, speckled penis, plantar keratosis, and facial and inguinal papillomas. Medical history includes a multinodular goiter, hypothyroidism, and the removal of colonic polyps at age 6 and 37 and two lipomas at age 37. Re-examination of the polyps was supportive of a diagnosis of CS. The proband is a college graduate and has no learning disabilities. His parents, his 3 children, and 2 sibs were evaluated. A nonsense mutation, Y16X, was found in the proband and his offspring, confirming this is a *de novo* mutation.

Y16X is the most proximal truncating mutation thus far reported in *PTEN*. We speculate his range of features is due to the location at the extreme 5end. CS/BRRS families have been reported but this is the first documented individual meeting clinical criteria for both CS and BRRS and adds further evidence that these are in fact different manifestations of the same disorder.

**ATM variants 7271TG and IVS10-6TG among women with unilateral and bilateral breast cancer.** *S.N. Teraoka<sup>1</sup>, J.L. Bernstein<sup>2</sup>, L. Bernstein<sup>3</sup>, W.D. Thompson<sup>4</sup>, S.L. Teitelbaum<sup>2</sup>, C.F. Lynch<sup>5</sup>, K.E. Malone<sup>6</sup>, J.D. Boice<sup>7</sup>, B.S. Rosenstein<sup>2</sup>, A.L. Børresen-Dale<sup>8</sup>, R.A. Gatti<sup>9</sup>, P. Concannon<sup>1</sup>, R.W. Haile<sup>3</sup>, WECARE Study Collaborative Group.* 1) Dept Molecular Genetics, Benaroya Research Institute, Seattle, WA; 2) Mount Sinai School of Medicine, New York, NY; 3) University of Southern California, Los Angeles, CA; 4) University of Southern Maine, Portland, ME; 5) University of Iowa, Iowa City, IA; 6) Fred Hutchinson Cancer Research Center, Seattle, WA; 7) International Epidemiology Institute, Rockville MD; 8) Norwegian Radium Hospital, Oslo, Norway; 9) University of California at Los Angeles, Los Angeles, CA.

Recent reports suggest that two ATM gene mutations, 7271TG and IVS10-6TG, are associated with a high risk of breast cancer among multiple-case families. To assess the importance of these two mutations in another genetically susceptible group, young women (under age 51) with bilateral breast cancer (second primaries), we compared the frequency of these mutations in the bilateral cases and in matched controls who had unilateral breast cancer. The 1149 women included were enrolled in an on-going population-based case-control study of the genetic factors that contribute to bilateral breast cancer; they were not selected on the basis of family history of cancer. Screening for 7271TG and IVS10-6TG ATM gene mutations was conducted using DHPLC followed by direct sequencing. The 7271TG mutation was detected in 1/638 (0.2%) women with unilateral breast cancer and in none of the bilateral cases (0/511), and the IVS10-6TG mutation in 8/638 (1.3%) unilateral and in 1/511 (0.2%) bilateral breast cancer cases. Carriers of either mutation were not limited to women with a family history. Given the likelihood that young women with bilateral breast cancer have a genetic predisposition, the observed mutation distribution is contrary to that expected if these two mutations were to play an important role in breast carcinogenesis in this population.

**SMAD4 tumor suppressor in early onset colorectal cancers.** *S.G. Royce<sup>1</sup>, K.J. Alsop<sup>1</sup>, A.M. Haydon<sup>3</sup>, M.A. Jenkins<sup>2</sup>, L.J. Mead<sup>1</sup>, G.G. Giles<sup>4</sup>, J.L. Hopper<sup>2</sup>, M.C. Southey<sup>1</sup>.* 1) Genetic Epidemiology Laboratory, Department of Pathology; 2) Centre for Genetic Epidemiology, Department of Public Health, The University of Melbourne; 3) Department of Epidemiology and Preventative Medicine, Monash University; 4) Cancer Epidemiology Centre, Cancer Council Victoria, Australia.

The most frequent cytogenetic alteration in colorectal cancer is the deletion of 18q21. One of the genes located in this region, SMAD4, is a downstream regulator in the TGF- signaling pathway. Loss of SMAD4 function causes loss of transcription of genes critical in cell cycle control. SMAD4 protein is reported to be absent in 38% of colorectal carcinomas and reduced in a further 28%. Furthermore, SMAD4 may assist the well-characterized tumor suppressor E-cadherin in the re-establishment of epithelial morphology. A recent study has shown that unselected colorectal carcinoma patients with normal SMAD4 diploidy have a three-fold higher benefit from 5-fluorouracil adjuvant chemotherapy than those with a deletion. The underlying genes and molecular pathways in the majority of early onset colorectal cancers remain undefined. We investigated the role of SMAD4 mutations in a population-based case study of early onset colorectal cancer. Immunohistochemistry for SMAD4, E-cadherin and -catenin was performed on invasive carcinomas from 106 participants diagnosed before the age of 45. SMAD4 protein showed nuclear, and occasionally, cytoplasmic localization in tumor epithelial cells, 26 cases exhibited loss of staining for SMAD4 protein, 10 of these with accompanying loss of cell adhesion proteins, and 7 showed focal positive and negative SMAD4 staining. Loss of SMAD4 was not associated with degree of tumor differentiation, however loss was related to tumor site and was higher in rectal than colonic carcinomas ( $p=0.03$ ). Loss therefore appears to be a common event in early onset colorectal carcinomas. Studying the role and nature of SMAD4 mutations relative to the established molecular pathways of early onset colorectal cancer, including those involving mismatch repair and K-ras mutations, has the potential to more effectively target treatment to patients.

**Modulation of p12DOC-1, a cell growth suppressor in in-vitro mismatch repair (MMR) human/mouse expression systems.** *Z.Q. Yuan<sup>1</sup>, T.S. Kent<sup>2</sup>, Y. Wang<sup>3</sup>, A.G. Miller<sup>2</sup>, W. Edelmann<sup>3</sup>, T.K. Weber<sup>1,2</sup>*. 1) Molecular Gen, Albert Einstein Col Medicine, Bronx, NY; 2) Surgery,; 3) Cell Biology, Albert Einstein Col Medicine, Bronx, NY.

**Introduction:** p12DOC-1, a CDK2-associated protein, is a candidate cell growth suppressor. We previously demonstrated that absent or decreased p12DOC-1 expression in multiple microsatellite-unstable (MSI+) colorectal cancer cell lines, or p12DOC-1 downregulation, was associated with colorectal epithelial cell proliferation and arrest of apoptosis. To better understand the relationship between p12DOC-1 expression and MMR status, we employed purified in vitro human and mouse recombinant MMR expression systems to investigate the relationship of p12DOC-1 expression to cell cycle and apoptosis regulation. **Methods:** Three MMR expression systems were studied, 1): A human MMR-deficient system was constructed by transfecting wild-type and mutant-type hMLH1 into the cell lines, 293T, RKO, and HCT116; 2): A human MMR-proficient system (HCT116+chromosome 3); 3): A mouse embryonic stem (ES) cell (MSH2-/- knockouts) MMR-deficient system. MMR status was confirmed via hMLH1 and hMSH2 assays. p12DOC-1 mRNA and protein expression were assessed in each system by real-time PCR and Western blot. The percentage of apoptotic cells and S-phase were determined by FACS assay. **Results:** In our MMR-deficient systems, the transcription and translation of p12DOC-1 were significantly decreased. Cell proliferation was increased and apoptosis decreased. In the MMR-proficient systems, p12DOC-1 transcription and translation were significantly increased compared to MMR-deficient controls. Cell proliferation was inhibited and apoptosis increased. Mouse expression system results were consistent with human system results. **Conclusion** Our current investigation of p12DOC-1 expression, cell cycle, and apoptosis in multiple MMR expression systems confirms our earlier findings with respect to p12DOC-1 expression and cell cycle and apoptosis modulation. We conclude that p12DOC-1 is an important regulator of cell cycle and apoptosis in human colorectal cancer.

**Ecogenetics of familial pancreatic cancer.** *A.E. Weedn<sup>1</sup>, C.E. Aston<sup>2</sup>, M.A. Grim<sup>1</sup>, D.E. Bard<sup>1</sup>, J.J. Mulvihill<sup>1</sup>.* 1) University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2) Oklahoma Medical Research Foundation, Oklahoma City, OK.

To identify the ecogenetic (gene-environment) determinants of pancreatic cancer through an interdisciplinary family studies approach, the National Registry of Pancreatic Cancer Families was established in 1988 at the National Cancer Institute. Registry families meet one of two inclusion criteria: 1) at least two first-degree relatives with adenocarcinoma of the exocrine pancreas, or 2) two affected second-degree relatives connected by a relative with any type of cancer. Family members complete questionnaires on demographics, medical history, and family history; documentation is obtained to confirm cancer history. Of the 78 eligible families with confirmed diagnoses, 5 were excluded as likely *BRCA1*, *BRCA2*, or *HPNCC* families. Aggregation analysis was performed on the 73 remaining families for pancreatic cancer as well as associated cancers by the method of Chakraborty et al (1984). All families aggregated for pancreatic cancer at a 5% level of significance, reflecting our inclusion criteria. Under the most stringent statistical conditions ( $P < .001$ ), aggregation of pancreatic cancer occurred in 17 families. Inspection of associated cancers in Registry families suggests possible excesses of other cancers. Specifically, 63 (expected = 28.8) cases of breast cancer, with 15 cases of breast cancer aged 50 years or less (3.5), were reported. Aggregation occurred in 34 of the families. The observations suggest that families with two or more pancreatic cancer cases may be at an increased risk to develop other cancers, especially of the breast. The analyses identify a subset of families for a candidate gene search; additional families continue to be enrolled.

**Functional characterization of new coactivators of the AF-1 domain of estrogen nuclear receptor.** *S. Solórzano-Vargas, A. Leon-Del-Rio.* Department of Molecular Biology and Biotechnology, Instituto de Investigaciones Biomédicas, UNAM, Mexico D.F., Mexico.

Estrogen receptor (ER) is a ligand dependent transcriptional regulator that mediates the effects of estrogen on growth, metabolism and cell differentiation. ER contains two distinct transactivation domains known as AF-1 and AF-2 domains located at N-terminus and C-terminus respectively. The transcriptional activity of ER has been explained in terms of the chromatin remodeling produced by coactivators recruited by the AF-1 domain such as SRC-1, CBP/p300 and pCAF. Unlike AF-2 domain, the AF-1 region has only been poorly characterized despite it has been linked to tamoxifen (TOT) resistance in patients with breast cancer. To understand the role AF-1 in human cells and breast cancer tumors we identified cell factors that regulate the activity of ER using AF-1 as a molecular bait in a yeast two-hybrid assay. We isolated 9 different cDNA fragments representing 8 different genes. Only two of these genes have been identified previously (E3KARP and MAZ-1). The rest of the clones were named transcriptional coactivator 1-6. Transient transfection of full length TC-1, TC-2, TC-3 and TC-4 cDNAs into human cells showed an increase in the ER mediated transactivation of 216%, 169%, 246% and 174% respectively. In contrast transfection of MAZ-1 showed a discrete transcriptional repression of ER. Cotransfection of well characterized coactivator of ER SRC-1 with either E3KARP, TC-1 or TC-2 increased the ER activity up to 450% with respect to control cells. These results suggest that the TC factors may regulate the ER activity by regulating the formation or activity of the chromatin remodeling complex in the AF-2 domain. Identification of proteins involved in the transcriptional activity of AF-1 could lead to a better understanding of the ER mediated transcriptional activation and the characterization of the mechanism of TOT resistance in patients with breast cancer.

**Role of Proliferating Cell Nuclear Antigen (PCNA) mutations in inducing microsatellite instability in gliomas.**

*A.A. Pillai*<sup>1</sup>, *H.V. Easwar*<sup>2</sup>, *R.N. Bhattacharya*<sup>2</sup>, *M. Banerjee*<sup>1</sup>. 1) Human Molecular Genetics, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram 695 014, Kerala, India; 2) SCTIMST, Thiruvananthapuram, Kerala, India.

Role of Proliferating Cell Nuclear Antigen (PCNA) mutations in inducing Microsatellite instability in gliomas. Anitha A1., H.V.Easwar<sup>2</sup>, R.N.Bhattacharya<sup>2</sup>, and M.Banerjee<sup>1</sup> | Rajiv Gandhi Center for Biotechnology, 2SCTIMST, Thiruvananthapuram. Glial tumors are the most common among the various Central Nervous System (CNS) tumors. Neoplastic transformation in the normal human brain occurs as a result of a series of genetic alterations. These multiple genetic changes gradually transform growth-limited cells into highly invasive cells. This is brought about by genes that positively control cell cycle checkpoints, which are targets for oncogenic activation, and negative regulators, such as tumour suppressor genes, that are targets for inactivation. Microsatellite Instability (MSI) is known to be a mutator phenotype for genomic instability in several cancers. Understanding the molecular details of cell cycle regulation and checkpoint abnormalities in cancer with respect to MSI pattern will provide insight into potential therapeutic strategies. We intend to evaluate the role of mutations in PCNA in causing MSI in gliomas, under the influence of stable expression of cell cycle genes. We assessed the microsatellite instability and mutations in the coding regions of PCNA, under the influence of homogenous genetic background of cell cycle genes, tumor suppressor genes and mismatch repair genes (PCNA p16, p21, p53, pTEN, hMSH2 and hMLH1) in various grades of gliomas. Under similar genetic background the astrocytic tumors of low grade show MSI in 26% of the loci, while malignant grades show MSI only in 13% of the tumors. Alterations in BAT26, D9S942 and D20S835 seem to be characteristic of astrocytic tumors. Under the unaltered expression profile of p16, p21, p53 and PCNA genes, MSI of microsatellites in proximity to PCNA could suggest for its role in MSI. Results indicate that MSI also seems to be an early event in the pathogenesis of Glial tumors.

**Screening for BRCA1 and BRCA2 germ line point mutations in 600 Italian families with breast and/or ovarian cancer: mutation prevalence according to phenotype.** *B. Pasini*<sup>1,2</sup>, *S. Manoukian*<sup>2</sup>, *V. Pensotti*<sup>2,3</sup>, *R. Crucianelli*<sup>2,3</sup>, *G.B. Spatti*<sup>2</sup>, *R.A. Conti*<sup>2</sup>, *T. Genovese*<sup>2</sup>, *B. Peissel*<sup>2</sup>, *L. Mariani*<sup>2</sup>, *M. Merola*<sup>2</sup>, *G. Casalis Cavalchini*<sup>1</sup>, *M.A. Pierotti*<sup>2,3</sup>, *P. Radice*<sup>2,3</sup>. 1) Dep. Genetics-Biology-Biochem., University of Turin, Turin, (Turin), Italy; 2) Istituto Nazionale Tumori, Milan, (Milan), Italy; 3) Istituto FIRC di Oncologia Molecolare, Milan, (Milan), Italy.

Six hundred (600) Italian families have been collected since 1991 at the National Cancer Institute of Milan eligible to BRCA1 and 2 mutation analysis. Inclusion criteria were: recurrence of breast and/or ovarian cancer in at least 2 family members, early onset cases of the latter tumors, multiple primary tumors in a single subject or male breast cancer. BRCA1 and 2 germ line point mutations were identified in 220 families (37%), including 194 "deleterious" mutations (32%) and 26 genetic variants of unknown biological effect (4.3%). The mutation detection rate for deleterious point mutations ranged between 24% in familial female breast cancer to 55% in familial breast and ovarian cancer, reaching 59% in familial ovarian cancer. Early onset or pre-menopausal breast cancer and multiple primary tumors were the most effective predictors of BRCA mutations identified in 40.4% of breast cancer cases arising below 36 years, 42.5% of bilateral breast cancers and 71% of women affected by both breast and ovarian cancer. The number of affected relatives did not influence significantly the mutation detection rate. BRCA1 and 2 point mutations (128 and 66 deleterious, respectively) were distributed along the whole coding sequence of both genes: in BRCA1 as well as in BRCA2 most of the mutations identified were unique (71% and 77.5%, respectively) or recurrent in a few families. Beside BRCA1 recurrent mutations at exon 5 cysteine residues and 5382insC, there were only small founder effects. Of note, among the 220 mutations identified, 52 (24%) were missense mutations with known (19) or unproven biological effect. The latter were assayed for their biological impact by using the SIFT software: 17 missense mutations were identified which potentially affect protein function.

**Association between the occurrence of MPNST and subcutaneous and internal neurofibromas in NF1.** *T. Tucker<sup>1</sup>, P. Wolkenstein<sup>2</sup>, JM. Friedman<sup>1</sup>*. 1) Medical Genetics, Univ British Columbia, Vancouver, BC, Canada; 2) Service de Dermatologie, Hôpital Henri-Mondor, Créteil, France.

Neurofibromatosis 1 (NF1) is a fully penetrant autosomal dominant cancer predisposition disorder with variable expressivity, making it difficult to predict which patients will be severely affected. Individuals with NF1 have a lifetime risk of about 10% of developing malignant peripheral nerve sheath tumours (MPNST) and most develop from pre-existing plexiform neurofibromas. Benign neurofibromas of various types develop in almost all people with NF1, but the number, size, and type varies greatly among patients. It is not known if an individual's risk for developing MPNST bears any relationship to the burden of benign neurofibromas. The Henri-Mondor Database contains information on 476 probands with NF1 diagnosed by the NIH Criteria. The average age is 33; 68% were between 10 and 39 years of age at last exam. A semi-quantitative estimate of the number of cutaneous neurofibromas and information on the presence or absence of subcutaneous and internal neurofibromas and MPNSTs were recorded for each patient. Internal tumours were identified by abdominal ultrasound, CT or MRI and X-ray, CT or MRI of the thorax. MPNSTs were diagnosed by biopsy. We used logistic regression to determine the association between each of these features with age as a covariate. MPNSTs were 14.3 times (95% confidence interval [CI] 2.7-77.7) more likely to be present in individuals with internal neurofibromas than in patients without internal neurofibromas. Individuals with subcutaneous neurofibromas were 5.3 (95% CI 2.0-14.3) times as likely to have internal neurofibromas and 3.4 (95% CI 1.3-8.8) times as likely to have a MPNST as patients without subcutaneous neurofibromas. Patients with both subcutaneous and internal neurofibromas were 17.3 (95% CI 2.6-87.5) times more likely to have a MPNST than a patient with neither. Cutaneous neurofibromas were not associated with any of the above mentioned tumours. The observation that MPNSTs are associated with subcutaneous neurofibromas and internal neurofibromas suggests that individuals with such benign tumours may warrant increased surveillance for MPNST.

**Cancer Screening Adherence in Breast/Ovarian Cancer Families.** *M.E. Wood<sup>1</sup>, C.I. Grossman<sup>2</sup>, W.C. McKinnon<sup>1</sup>, B.M. Dausch<sup>2</sup>, A. Gray<sup>2</sup>, B.E. Compas<sup>3</sup>*. 1) Medicine, University of Vermont, Burlington, VT; 2) Psychology, University of Vermont, Burlington, VT; 3) Psychology, Vanderbilt University, Nashville, TN.

Women with strong family histories of breast and/or ovarian cancer have elevated ovarian cancer risk. This study evaluates adherence to ovarian cancer screening in high-risk women. Participants included women with a family history of breast and/or ovarian cancer who were recommended screening for ovarian cancer. Women were asked to complete questionnaires regarding medical and family history, health behaviors, cancer screening practices, psychological distress, adherence and barriers to ovarian cancer screening at study enrollment, 12 and 24 months. Forty-four women were enrolled, 5 had oophorectomy, 9 did not complete all time points and 1 patient expired; 29 women completed the study and are the focus of this report. The mean age of women in this study is 45 (range 29-71), 48% had breast cancer and 10 have BRCA mutations. We divided adherence into three groups; non-adherent (no exams over study period), minimally adherent (at least one CA125 or TVUS per year) and maximally adherent (exams every 6 months). Twenty eight percent of women did not adhere to any screening, 52% were minimally adherent and 20% were maximally adherent. Reasons for lack of adherence were most commonly related resources and estimation of risk. The most common barrier was the primary care providers failure to endorse screening. Variables associated with maximum adherence include intrusive thoughts and family history of ovarian cancer. Non-adherence was associated with avoidant thoughts related to cancer specific distress. Psychological distress in this group was comparable to a sample of women with newly diagnosed breast cancer, with greater levels of distress among unaffected women. We have shown a high-rate of adherence to ovarian cancer screening in this population in contrast to prior reports. Reasons for lack of adherence largely relate to PCP lack of recommendation. Having a family history of ovarian cancer was associated with some level of adherence to screening recommendations. Intrusive thoughts were associated with adherence while avoidant thoughts were associated with lack of adherence.

**The Protein Kinase A (PKA) Regulatory Subunit RI (PRKARIA) Alters MAP Kinase and Cell Cycle Entry in Transformed B-Lymphocytes.** *A.J. Robinson-White<sup>1</sup>, W. Leitner<sup>2</sup>, L. He<sup>3</sup>, D. Olson<sup>3</sup>, C.A. Stratakis<sup>1</sup>.* 1) UGEN/DEB, NICHD, NIH, Bethesda, MD; 2) DB, NCI, NIH, Bethesda, MD; 3) IRP AB, NIAMS, NIH, Bethesda, MD.

Carney complex (CNC) is associated with mutations in PRKAR1A, a gene that codes for the RI regulatory subunit of the cAMP-dependent protein kinase (PKA). A partial or complete loss of PRKAR1A apparently results in tumorigenicity, by an unknown mechanism. In many cells, RI inhibits the extracellular receptor kinase cascade (ERK1/2) of the MAP kinase (MAPK) signaling pathway causing decreased cell proliferation. In previous studies, we showed that upon stimulation of MAPK by lysophosphatidic acid (LPA, 0-150 nM), and PKA by isoproterenol and forskolin (0-100 uM), inhibition and stimulation of ERK1/2 phosphorylation occurred in normal and mutant cells, respectively. Isoproterenol and forskolin also inhibited LPA-induced cell proliferation and metabolism in normal cells, but caused stimulation in mutant cells. In the present studies, we asked whether the effects of PKA on ERK1/2 phosphorylation, cell proliferation and metabolism are via a decrease in cell cycle activity or by a stimulation of apoptosis. In normal cells, cell number decreased (2-60%) with isoproterenol, with no corresponding increase in cell death. Cell death was constant at 0-5% and no apoptosis was seen in normal or mutant cells (60-100 uM isoproterenol), as determined by TUNEL assay. In the same cell population, apoptosis could be induced by other stimuli (APO-1 and CH11, antiFAS/CD95 antibodies) showing 36-54% apoptosis. Phosphorylation of other components of the ERK1/2 cascade (c-Raf-1 and MEK1/2) was decreased by PKA stimulants in a similar manner to that of ERK1/2 in normal cells. Phosphorylation of the nuclear transcription factor c-Myc was also decreased in normal cells, but increased in mutant cells. We conclude: 1) PKA stimulation has opposite effects on ERK1/2 activity and cell growth and proliferation in mutant PRKAR1A vs normal transformed B-lymphocytes; 2) these changes are not associated with differences in apoptosis or in changes in DNA content of the cell cycle and 3) PKA may alter cell proliferation and metabolism in B-lymphocytes by altering the rate of entry of cells into the cell cycle.

**Colonoscopy utilization before and after HNPCC genetic testing.** *S.K. Peterson<sup>1</sup>, E.R. Gritz<sup>1</sup>, S.W. Vernon<sup>2</sup>, C.A. Perz<sup>3</sup>, S.K. Marani<sup>1</sup>, B.G. Watts<sup>1</sup>, C. Amos<sup>1</sup>, M. Frazier<sup>1</sup>, P.M. Lynch<sup>1</sup>.* 1) UT MD Anderson Cancer Center, Houston, TX; 2) UT Health Science Center School of Public Health, Houston, TX; 3) Univ Houston-Victoria, Victoria, TX.

Genetic testing can identify HNPCC-predisposing mutations in persons with a family cancer history suggestive of this syndrome. Mutation carriers have up to an 85% lifetime risk of developing colorectal cancer (CRC), and are advised to have annual or biannual colonoscopy usually beginning at age 25 years. In the absence of genetic testing, persons who are at increased risk for HNPCC by virtue of their family cancer history also are advised to follow the same cancer screening guidelines as mutation carriers. As part of a longitudinal study of psychosocial aspects of HNPCC genetic counseling and testing, we evaluated colonoscopy utilization before and after genetic testing. Participants were 79 adults who had no prior history of CRC and who were from families with a known HNPCC-predisposing mutation. Before pre-test genetic counseling, 53% (n=42) had ever had a colonoscopy. All participants underwent genetic testing, and 33% (n=26) were identified as carrying an HNPCC-predisposing mutation. There was no difference in baseline prevalence of colonoscopy use between mutation carriers and non-carriers (50% and 54%, respectively). Within 6 months of receiving test results, 76% of mutation carriers reported that they had undergone colonoscopy since receiving their results, a significantly greater proportion compared with baseline utilization and with non-carriers (F=18.87, p<0.001). Compared with baseline responses, mutation carriers also expressed significantly greater self-confidence in their ability to follow through with colonoscopy, a greater level of commitment to having colonoscopy, and fewer perceived barriers to CRC screening after test results disclosure. Undergoing HNPCC genetic counseling and testing may motivate mutation carriers to have a colonoscopy within a relatively short time period after disclosure of test results. HNPCC genetic counseling and testing also may positively influence mutation carriers' attitudes toward CRC screening.

**Cancer variation associated with the position of BRCA2 gene mutation.** *C.M. Phelan<sup>1</sup>, J. Lubinski<sup>2</sup>, P. Ghadirian<sup>3</sup>, H.T. Lynch<sup>4</sup>, J. Garber<sup>5</sup>, B. Weber<sup>6</sup>, D. Horsman<sup>7</sup>, J. Aube<sup>1</sup>, P. Sun<sup>1</sup>, S. Narod<sup>1</sup>.* 1) Center for Research in Womens' Health, 790 Bay St., Toronto, Ontario, Canada; 2) Hered Cancer Center, Pomeranian Academy of Medicine, Poland; 3) Unit de Recherche du CHUM, Htel Dieu, Montreal, Quebec; 4) Creighton University, Omaha, Nebraska; 5) Dana-Farber Cancer Institute, Boston, MA; 6) University of Pennsylvania, Philadelphia; 7) BC Cancer Agency, Vancouver, BC, Canada.

Inherited mutations of the BRCA2 gene give rise to a multi-site cancer phenotype which includes breast cancer, ovarian, pancreatic and prostate cancer, melanomas, laryngeal, colon and stomach cancers. Interpretation of test results and risk assessment is therefore complex. It has been proposed that families with mutations in the ovarian cancer cluster region (OCCR) of exon 11 (nucleotides 3035-6629) expressed a higher ratio of ovarian to breast cancer, than families with mutations elsewhere in the BRCA2 gene. In this study we have investigated the presence of seven types of cancer (ovary, male breast, pancreas, prostate, colon, stomach and melanoma) in 419 families with a BRCA2 mutation. Families with ovarian cancer were more likely to harbour mutations in the OCCR (nucleotides 3035-6629) compared to the remainder of the gene (OR = 2.20; p=0.0003). Ashkenazi Jewish families with the 6174delT founder mutation were more likely to have a family member with ovarian cancer (O.R. = 1.63, p=0.0018) and less likely to have a family member with prostate cancer (OR = 0.55; p=0.017) than were families of other ethnic origins. In contrast, a reduced presence of ovarian cancer was found with families of French-Canadian ancestry, compared to other ancestries (O.R. = 0.37, p=0.0026). Male breast cancer risk was observed with the French-Canadian founder mutation 6503delTT (OR = 15.0; p = 0.026). Families of Polish ancestry were associated with a reduced frequency of prostate cancer (OR = 0.29; p = 0.05) and of colon cancer (OR = 0.12; p=0.009) compared to families of other ethnic origins. In conclusion, both the position of mutation in BRCA2 and the ethnic background appear to contribute to the phenotypic variation observed in families with these mutations.

**Genetic polymorphism in the androgen receptor and prostate-specific antigen genes and prostate cancer risk.**

*C.A. Salinas<sup>1</sup>, M. Langlois<sup>2</sup>, L. Iwasaki<sup>1</sup>, E.A. Ostrander<sup>2</sup>, J.L. Stanford<sup>1</sup>.* 1) Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA.

Prostate cancer is an androgen-dependent disease, with the androgen receptor (AR) regulating transcription of androgen-responsive genes in the prostate. Polymorphic CAG and GGN repeats in exon 1 of the AR may alter transcriptional activity, potentially influencing prostate cancer risk. A recent study suggests that short CAG alleles increase prostate cancer risk 5-fold in men homozygous for the G allele (-189) in the prostate-specific antigen (PSA) gene. To further evaluate the association of these polymorphisms with prostate cancer, we conducted a population-based study of Caucasian men. Germline DNA was collected from 553 newly diagnosed prostate cancer cases, aged 40 to 64 years, from King County, WA. 520 population-based controls were identified and frequency-matched to cases by age. Information on potential risk factors was collected by in-person interviews. Logistic regression was used to estimate odds ratios (OR) and assess statistical significance. Linear regression models were fit to estimate the effect of AR and PSA genotypes on serum PSA levels in controls. No association was detected between prostate cancer and having 22 compared with having 22 CAG repeats (OR=1.1; 95% CI 0.9-1.4), having other than 16 GGN repeats (OR<16 repeats =1.0; 95% CI 0.7-1.4; OR>16 repeats =0.9; 95% CI 0.7-1.3), or having the variant PSA AA at the PSA promoter compared with the PSA GG genotype (OR=1.0; 95% CI 0.7-1.4). Geometric mean serum PSA levels in men having 22 CAG repeats and PSA GG were 0.3% higher (95% CI -23% - +30%) than men with 22 repeats and PSA not GG genotype. No association was detected between disease aggressiveness and any of the AR or PSA genotypes. No evidence was found that risk of prostate cancer associated with CAG or GGN repeats varied within subgroups of the PSA genotype, family history of prostate cancer, or body mass index. The present study suggests that these AR and PSA genetic polymorphisms are not associated with risk of prostate cancer in middle-aged men.

**A paucity of identifiable mismatch repair gene mutations following a comprehensive screening of HNPCC-like families from Newfoundland.** *M.O. Woods<sup>1,2</sup>, J.S. Green<sup>2</sup>, D. Robb<sup>3</sup>, A. Pollett<sup>1</sup>, B. Younghusband<sup>2</sup>, J. McLaughlin<sup>4</sup>, P.S. Parfrey<sup>5</sup>, B. Bapat<sup>1</sup>.* 1) Lab Medicine & Pathobiology, Univ Toronto, Toronto, ON, Canada; 2) Genetics, Memorial University of Newfoundland, St. John's, NL, Canada; 3) Pathology, Memorial University of Newfoundland, St. John's, NL, Canada; 4) Public Health Sciences, Univ Toronto, Toronto, ON, Canada; 5) Nephrology, Memorial University of Newfoundland, St. John's, NL, Canada.

Colorectal cancer (CRC) is the second leading cause of cancer-related death in the United States. Hereditary Non-Polyposis Colorectal Cancer (HNPCC) is the most common form of hereditary colorectal cancer comprising between 1-10% of all CRC cases. Mutations in at least five mismatch repair genes are known to cause HNPCC. Approximately 95% of the disease causing alterations are found in MLH1, MSH2 and MSH6. Twenty-seven high risk Newfoundland HNPCC-like families were assessed to determine Mismatch Repair (MMR) alterations, cancer risks and possible genotype-phenotype correlations. In order to determine mismatch repair deficiencies, a comprehensive screening of probands was undertaken. When available, tumour tissue was assessed for MLH1, MSH2 and MSH6 presence/absence using immunohistochemistry. As well, microsatellite instability (MSI), a hallmark of HNPCC, was evaluated on all available tumours using markers BAT-25 and BAT-26. To assess genomic alterations, automated sequencing of all exons and intron/exon boundaries was performed for MLH1, MSH2 and MSH6 on every proband. Additionally, a relatively new technique called Multiplex Ligation-dependent Probe Amplification (MLPA), was used to identify genomic deletions in MLH1 and MSH2. Surprisingly, few disease-causing alterations were identified in our scan of these high risk families. Most notably, a deletion of MSH2 exon 8 was identified in four families from the same geographical area on the island. Also, two novel alterations of interest occurred in MLH1 (V49E and G22A); each in a single family. Due to the paucity of mutations thus far identified using a comprehensive screening procedure, some of these large kindreds could be valuable in mapping additional genes causing HNPCC-like disease.

**A novel technique for detecting genomic copy number changes combined with genotyping using oligo arrays.**

*G.R. Bignell<sup>1</sup>, J. Huang<sup>2</sup>, M.H. Shapero<sup>2</sup>, K.W. Jones<sup>2</sup>, W. Wei<sup>2</sup>, H. Zang<sup>2</sup>, A.P. Futreal<sup>1</sup>, M.R. Stratton<sup>1</sup>, R. Wooster<sup>1</sup>.*  
1) Cancer Genome Project, Sanger Ctr, Hinxton Cambridge, United Kingdom; 2) Genotyping Research, Affymetrix Inc, 3380 Central Expressway, Santa Clara CA95051, USA.

We carried out analysis using an oligo array to detect copy number changes in cancer cell lines, to date over 200 cancer cell-lines have been screened. Using the protocol developed we were able to detect homozygous deletions, gene amplifications and regions of reduced and elevated chromosomal copy number. The statistical significance (p-value) of any genomic alteration is given for each data point. The results from this type of analysis are similar to those of array CGH but have the added advantage of the associated genotype analysis for each data point allowing for a more thorough interpretation of the underlying alterations. Validation experiments to demonstrate the change in fluorescence in response to underlying changes in genomic copy number are also presented with the analysis of lines containing 1 to 5 copies of the X-chromosome, together with experiments where the copy number of 42 SNPs were increased up to 1000 fold by spiking with PCR products containing the relevant DNA sequence. These results indicate that at lower copy number (1 to 5 fold) the increase in fluorescence in response to increased copy number gives a linear response ( $R^2$  0.9967) which, although not directly proportional, is fairly good (5 copies gives an estimated value of 4.1), at higher copy number (up to 1000 fold) the relationship between copy number and fluorescence is linear although not directly proportional when plotted on a log-log scale. Further validation of the technique has been carried out confirming a selection of those genomic alterations detected, confirmation of genomic amplifications was carried out using real time PCR and homozygous deletions by PCR visualised by gel electrophoresis.

**No evidence of recurrent BRCA1/2 genomic rearrangement in high risk French-Canadian breast/ovarian cancer families.** *A.M Moisan, J. Fortin, M. Dumont, C. Samson, J. Simard, INHERIT BRCA's.* Cancer Genomics Laboratory, CHUL Research Center, Quebec, Canada.

The discovery of two genes, BRCA1 and BRCA2, which, when altered, confer markedly increased susceptibility to breast and ovarian cancer, has facilitated the identification of individuals at particularly high risk of these diseases. However, the literature shows wide variations in the prevalence of BRCA1/2 mutations and their related risk from one population to another and these estimates may not be extrapolated to Canadians, especially to founder populations, such as the French Canadians. The majority of these mutations were detected using PCR-based methods. These approaches do not allow the detection of large DNA rearrangements, which have been claimed to be involved in other populations in 5 to up to 36% of BRCA1 positive families, whereas there is very little, if any, information about the contribution of this type of mutations in BRCA2 positive families. To investigate if our available mutation spectrum of BRCA1 and BRCA2 in high risk French Canadian breast/ovarian cancer families has been biased by PCR-based direct sequencing method, DNA samples from 94 affected/obligate carrier individuals from 87 families (including 26 BRCA1/2 positive families as controls) were tested by Southern blot analysis. Moreover, the absence of rearrangement involving smaller exons have been verified by PCR using cDNA samples. Three RFLPs have been observed in BRCA1, whereas six were found in BRCA2. In BRCA1, a characterized polymorphism in exon 16 has been observed in 55 individuals, whereas 2 individuals has a supplementary bands with *AvaII* and *BglIII*, which is due to the high sequence similarity of exon 24 and a portion of chromosome 4. In BRCA2, a polymorphism have been observed in intron 16 abolishing a *AvaII* site in 25 individuals, whereas a new *TaqI* restriction site in the 3'UTR has been found in one sample. Based on our current extensive analysis, there is no evidence supporting the existence of any deleterious BRCA1/2 recurrent genomic rearrangement in the French-Canadian breast/ovarian population and we will validate these results for BRCA1 with the new Multiplex Ligation-dependent Probe Amplification technique.

**Whole Genome DNA Copy Number Changes Identified by Oligonucleotide Arrays.** *K.W. Jones<sup>1</sup>, J. Huang<sup>1</sup>, W. Wei<sup>1</sup>, H. Zhang<sup>1</sup>, G.R. Bignell<sup>2</sup>, A.P. Futreal<sup>2</sup>, M.R. Stratton<sup>2</sup>, R. Wooster<sup>2</sup>, M.H. Shapero<sup>1</sup>.* 1) Molecular Genetics, Affymetrix, Inc., Santa Clara, CA; 2) Cancer Genome Project, Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK.

Genetic instability, such as changes in DNA copy number, is one of the hallmark features of most human cancers. We have developed a method termed whole genome analysis (WGA) which can genotype over 10,000 single nucleotide polymorphisms (SNPs) from human genomic DNA and can be used to identify DNA gains and losses across all chromosomes. This assay uses a single primer to amplify representative fractions of the genome followed by SNP genotyping via hybridization to high density oligonucleotide arrays. We have developed algorithms that jointly use PM intensity and discrimination ratios between paired perfect match (PM) and mismatch (MM) intensity values to identify and estimate genetic copy number change. A chip-wise normalization is performed to improve comparability across samples. SNP discrimination ratios and PM intensities from an experimental sample are then compared to distributions derived from a reference set containing over 100 normal individuals, allowing statistically significant regions of DNA copy number changes to be identified. Based on Taqman results from an established human breast cancer cell line, this approach shows good sensitivity and specificity. In addition, statistically significant genomic intervals showing loss of heterozygosity (LOH) can be identified by calculating the likelihood of a contiguous stretch of homozygous markers based on known allele frequencies using the same reference set of normal individuals. With mean and median inter-SNP distances of 241 kb and 115 kb respectively, this method affords resolution that is not easily achievable with alternative experimental approaches.

**Screening of 75 patients for 7 translocations by Multiplex RT-PCR.** *A.H. Taronchi, R. Karimi nejad, M. Zanganeh, H. Imanian, T. Nayeb bagher, S. Niazi, H. Najmabadi.* Karimi nejad Najmabadi Pathology & Genetics Center.

We have introduced a multiplex reverse transcription-Polymerase chain reaction (RT-PCR) method for diagnosis and screening of patients with 7 chromosomal translocations including: t(1;19)(q23;p13), t(12;21)(p13;q22), inv(16)(p13;q22), t(15;17)(q21;q22), t(9;22)(q34;q11), t(8;21)(q22;q22), t(4;11)(q21;q23). Many of these translocations are related to acute and chronic, myeloid and lymphoid leukemias. Multiplex RT-PCR detects hybrid mRNAs transcribed from the fusion genes produced by translocations. We have studied 75 patients with the following indications and results:

	Total number	Positive for translocation	Type of translocation
CML	43	21	t(9;22)
AML-M4 or M2	7	2	inv(16),t(8;21)
AML-M3	12	8	t(15;17)
ALL	10	1	t(9;22)
other	3	0	0

BCR/ABL b2a2 and b3a2 were the most frequently detected breakpoints in t(9;22)translocations.57 cases of 75 patients had cytogenetic analysis simultaneously. In 3 cases cytogenetic analysis failed in detection, because of insufficient metaphase cells but RT-PCR analysis revealed t(15;17) fusion gene RNAs. Also 3 case of inconclusive cytogenetic analysis were positive for t(9;22) in our method. Also cytogenetic analysis showed other numerical and structural aberrations in 3/57 of cases.

**Telomerase activation and telomere length in head and neck cancer in Western India (Gujarat): Institutional experience.** *R.M. Rakesh, R.N. Sainger RN,, P.M. Shah, P.S. Patel.* Biochemistry Research Division, Department of Cancer Biology, The Gujarat Cancer & Research Institute, Ahmedabad-380016, Gujarat, India.

During replication of the linear chromosomes, telomeres, i.e. the ends of the chromosomes, are not replicated completely by the conventional DNA polymerases. Therefore, normal somatic cells senesce after certain number of cell divisions. Telomerase is a special reverse transcriptase used by most eukaryotes to achieve immortalization. Telomerase activity has been determined in a variety of cancers. However, there are few reports on telomerase activity in head and neck cancer. The etiology of the disease in India is completely different from Western countries. Tobacco consumption is more prevalent in India and the mode of tobacco consumption (e.g. chewing, snuffing, bidi smoking, reverse smoking) is also different. Telomerase activation and telomere length alterations were studied in tumor and adjacent normal tissues in 110 patients with head and neck cancer and 40 patients with precancerous/benign conditions. Telomerase activity and telomere lengths were determined by Telomeric Repeat Amplification Protocol (TRAP assay) and Southern blot analysis, respectively. **RESULTS:** Telomerase activation was observed in 78.2% of the malignant tissues, 85% of the precancerous tissues, and 53.1% of the adjacent normal tissues. Peak terminal restriction fragment length (TRF) was observed to be significantly lower in malignant tissues compared with the adjacent normal tissues. No significant correlation could be observed between telomerase activation and clinicopathologic characteristics of the patients. Two-year disease-free survival analysis showed that patients showing telomerase activation in the adjacent normal tissues and patients showing higher telomere length in malignant tissues had poor disease-free survival. Our results demonstrate the significant clinical usefulness of telomerase activation and telomere length for head and neck cancer patients. These markers may be helpful in predicting the clinical course of the disease and thus in identifying the patients in need of a close follow-up and vigorous adjuvant treatment. Email: rakeshmrawal@yahoo.com.

**Comparing Familial Aggregation Study Designs: The Long Island Breast Cancer Study Project.** *R. Zimmerman<sup>1</sup>, H. Ahsan<sup>2</sup>, M. Gammon<sup>3</sup>, D. Greenberg<sup>1</sup>*. 1) Div.Statistical Genetics, Dept.Biostatistics, Columbia Univ., New York, NY; 2) Depart.Epidemiology, Columbia Univ, New York, NY; 3) Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC.

The Long Island Breast Cancer Study Project (LIBCSP) is a population-based case-control study of breast cancer on Long Island. The project data allow us to compare empirically two familial aggregation study designs, results of which reflect the genetic influence on a disease. These data also allow us to determine the familial risk of breast cancer on Long Island, New York. Over the last decade, investigators suggested that case-control derived effect estimates were biased compared to reconstructed cohort effect estimates. The perception was also wide-spread that confounding by family size and age further undermined the case-control design validity. However, our recent comparison of familial aggregation study designs demonstrated that the reconstructed cohort poses no benefit over the case-control design. Furthermore, we showed that the accuracy of estimates of genetic predisposition is a function of the family history measure used, as well as the prevalence of disease and the proportion of genetic disease in the populations. Based on the above work, we were able to estimate that effect estimates derived a first-degree relative family history measure (as used in the case-control design) would be slightly larger or comparable to a single relative family history measure (as used in the reconstructed cohort) when analyzing the LIBCSP breast cancer data. We found that the familial aggregation based on case-control suggested slightly more familiarity than that based on reconstructed cohort. Though family history is a poor measure of genetic risk, different measures of family history may provide more information about underlying genetic risk than previously understood.

**Missense mutations in cancer suppressor gene TP53 are associated with Exonic Splicing Enhancers (ESEs).** *I. Gorlov, O. Gorlova, M. Frazier, C. Amos. Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX.*

We studied the association of exonic splicing enhancers (ESEs) with missense mutations in the tumor suppressor gene TP53 using the International Agency for Research on Cancer (IARC) mutation database. The goal of the study was to identify potentially functional ESE sites in the open reading frame (ORF) of the TP53. If a sequence functions as a splicing enhancer, then nucleotide substitutions in the site will disturb splicing, abrogate p53 function, and cause cancer. Therefore, functional ESE sites are expected to show increased number of missense mutations in affected individuals. Using several statistical tests, we found that missense mutations in TP53 strongly co-localize with ESEs, and that only a small fraction of ESE sites contributes to the association. There are usually 1 or 2 ESEs per exon showing a statistically significant association with missense mutations - so-called significant ESE sites. In many respects significant ESE sites are different from those that do not show association with missense mutations. In most exons, there are qualitative differences between significant and nonsignificant ESEs in the number of mutations. We also found that positions of significant ESE sites are codon-dependent - significant ESEs preferentially start from the first position of a codon. Individual significant ESEs show more consistent sequences compared to nonsignificant ESEs. These findings suggest that the ESE sites harboring significantly elevated number of missense mutations are functional splicing enhancers.

**CDKN2a Mutation Database.** *M. Greenblatt, J. Murphy, R. Barrantes-Reynolds, J. Bond.* Vermont Cancer Ctr, U Vermont, Burlington, VT.

Databases of gene variation can be valuable tools in studying the relationship of gene mutation to disease. In cancer genetics, database analysis can help clinicians and researchers interpret the significance of allelic variants. There are many databases of germline genetic variants that predispose to cancer, but few somatic mutation databases for cancer-related genes. We present a database of mutations in the CDKN2a gene (Ink4a, p16), available to the scientific community through the WWW ([www.biodesktop.uvm.edu/perl/p16](http://www.biodesktop.uvm.edu/perl/p16)). CDKN2a is mutated in many cancers and is responsible for some cases of Familial Melanoma. Our database improves upon existing resources by: 1) including somatic and germline variants, adding the perspective of somatic cell carcinogenesis to that of hereditary cancer predisposition; 2) annotation with information that may assist with the interpretation of allelic variants. This includes data regarding techniques of mutation analysis, intragenic evolutionary patterns, protein structure, and in vitro functional studies. We include correlation of these features with disease-associated mutations, statistical validation, and figures illustrating our findings and conclusions; 3) a format that allows a user to either download the database as an Excel file or to manipulate it online. Mutation and functional data were compiled by searching PubMed through Dec 2002. Functional, evolutionary and structural data are included from our recent paper (Greenblatt et al, *Oncogene* 22:1150-1163, 2003). We present the database through the UVM BioDesktop, a web infrastructure that combines features of a conventional desktop operating system, such as a graphical folder hierarchy, with those of a web-based biological database. The CDKN2a database is part of a larger project whose objectives are to develop and test a model for predicting the biological consequences and clinical relevance of CDKN2a missense variants by detailed study of gene evolution, disease-associated mutations, and protein structure and function. We hope to use CDKN2a as a prototype for integrating computational and laboratory data to help interpret variants in other cancer-related genes and single nucleotide polymorphisms (SNPs) found throughout the genome.

**The distribution of constitutional *NF2* mutations: results from an international *NF2* database.** *M.E. Baser*<sup>1</sup>, *A.J. Wallace*<sup>2</sup>, *S. Olschwang*<sup>3</sup>, *E. Bijlsma*<sup>4</sup>, *L. Papi*<sup>5</sup>, *D.M. Parry*<sup>6</sup>, *G.A. Rouleau*<sup>7</sup>, *D.G.R. Evans*<sup>2</sup>. 1) Los Angeles, CA, USA; 2) Department of Medical Genetics, St. Mary's Hospital, Manchester, UK; 3) INSERM U434, Fondation Jean-Dausset-CEPH, Paris, France; 4) Department of Clinical Genetics, Academic Medical Centre, University of Amsterdam, The Netherlands; 5) Department of Clinical Physiopathology, University of Florence, Italy; 6) Genetic Epidemiology Branch, National Cancer Institute, Bethesda, MD, USA; 7) Center for Research in Neuroscience, McGill University, Montreal, Canada.

A number of studies have suggested that *NF2* mutations are more common in exons 1-8 than in exons 9-15. We tested this hypothesis in an *NF2* database that has constitutional *NF2* mutations from published studies, from the United Kingdom *NF2* registry, and from unpublished data that are contributed by investigators. The study had 397 families with microdeletions in *NF2* coding sequences (excluding 134 families with splice-site mutations, 48 families with large deletions, and four families with chromosomal translocations). CpG dinucleotides are mutational hotspots in humans, and there are seven *NF2* codons where a CT transition would change an arginine codon (CGA) to a stop codon (TGA). One hundred twenty (58%) of the 205 nonsense mutations occurred in these codons. Mutations were more common in CGA codons 57, 196, 198, and 262 in exons 1-8 (N = 84) than in codons 341, 466, and 588 in exons 9-15 (N = 36). There were no reported mutations in codon 588. The seven *NF2* sites where GAG and GAA codons are preceded by a C also may be prone to transition to C:TAG and C:TAA due to a CpG on the opposite strand, but there were only six mutations at these sites, three of which were 1387 GT. After excluding CT transitions in CGA codons, the frequency of micro-lesions in the FERM domain (codons 1-302) was 0.51/bp (SE, 0.04/bp); in the -helical domain (codons 303-478), 0.47/bp (SE, 0.06/bp); and in exons 14 and 15 (codons 483-579) of the C-terminal domain, 0.34/bp (SE, 0.08/bp) (mutations have not been reported in exons 16 or 17). We conclude that the uneven distribution of micro-lesions in *NF2* coding sequences is due to CT transitions in CGA codons.

**Towards the definition of the cancer transcriptome.** *D.S. Gerhard<sup>1</sup>, R.L. Strausberg<sup>1</sup>, G.J. Riggins<sup>2</sup>, C.F. Schaefer<sup>3</sup>, L. Wagner<sup>4</sup>, V. Jongeneel<sup>5</sup>, A. Simpson<sup>6</sup>.* 1) Office of Cancer Genomics, NCI/NIH, Bethesda, MD; 2) Duke University, Durham, NC; 3) Center for Bioinformatics/NCI; 4) NCBI/NIH; 5) Ludwig Institute for Cancer Research, Louisanne, Switzerland; 6) Ludwig Institute, NY, NY.

Among the important questions still unanswered is the transcriptome size and composition of normal and transformed cells. We focus our efforts towards determining the answer by a number of ways. One is serial analysis of gene expression that was performed on 138 cells, normal and transformed tissues and cell lines to the depths of approx. 50,000 tags per cell as part of the Cancer Genome Anatomy Project (CGAP). More samples will be added within the next few months. From the data we can conclude that on the average ~19,000 genes are expressed in normal cells and that number does not change substantially in transformed cells. In general, the number of genes per sample increases somewhat with increased number of tags obtained. CGAP has a web viewer where the difference of expression levels for any known gene in normal and tumors cells can be determined visually as well as numerically. These data are found at <http://cgap.nci.nih.gov>. Recently, we used the massively parallel signature sequencing to generate tags and obtained greater than a million tags per cell line. The predicted size of the transcriptome was essentially unchanged. Another tool that provides cellular transcription information is the generation of full-length cDNAs. The Mammalian Gene Collection (MGC) generates tissue- and tumor-specific cDNA libraries and full length, sequenced clones for new and known genes for both the mouse and human. To date, there are clones for 11,276 non-redundant human and 7,940 mouse genes. Recently, we have added libraries from 5 different ES cell lines and expect to identify the ES-specific genes and isolate full length clones. All clones are accessible to the scientific community and can be viewed at the MGC web site <http://mgc.nci.nih.gov/>. The genes for which clones are available fall into many pathways, tissue types and transcription levels. The lists of member of the CGAP and MGC projects can be found on the web sites.

**Cancers as a complex phenotype.** *L.T. Amundadottir<sup>1</sup>, S. Thorvaldsson<sup>1</sup>, U. Thorsteinsdottir<sup>1</sup>, H. Sigurgeirsson<sup>1</sup>, S. Arnason<sup>1</sup>, K. Kristjansson<sup>1</sup>, A. Kong<sup>1</sup>, M. Frigge<sup>1</sup>, J. Bjornsson<sup>2</sup>, J.R. Gulcher<sup>1</sup>, K. Stefansson<sup>1</sup>.* 1) Department of Cancer Genetics, deCODE genetics, Reykjavik, ICELAND; 2) Department of Pathology, National University Hospital, Reykjavik, ICELAND.

Numerous linkage studies have unraveled genetic factors explaining cancer syndromes where highly penetrant mutations increase the risk of one or few types of cancers in families. However, these only explain a minority of all cancer cases. Inherited predisposition for the majority of cancer cases probably involves the interaction of multiple low risk genes where different combinations affect the site and severity of the disease. In an attempt to find evidence for cancer sites with shared genetic factors we have studied the familiarity of all cancer cases in Iceland. This was done by linking the entire Icelandic Cancer Registry containing all cancer patients diagnosed in Iceland from 1955-2002 to an extensive genealogical database containing all living Icelanders and most of their ancestors since the settlement of Iceland in the 9th century. This allowed us to estimate risk ratios (RR) and kinship coefficients (KC) both within and between all cancer sites. The completeness of the genealogical database furthermore allowed us to study relationships up to 5th degree making our approach unique in its ability to assess the effects of familiarity outside the nuclear family where individuals are less likely to share environmental factors but still share a substantial amount of their genetic makeup. Both risk and kinship calculations indicated that breast and prostate cancer have the highest level of extended familiarity and the most noticeable cluster of linked cancer sites included breast, prostate and kidney cancer. Other clustering will be presented. Our results indicate that not only are genetic factors involved in the etiology of cancers of specific sites but also that shared genetic factors are important in the context of cancers at combinations of specific sites. Furthermore, in some instances the difference between cancers at different sites may be explained by variation in expressivity rather than involvement of different susceptibility genes.

**Familial Risk of Lung Carcinoma in the Icelandic Population.** *U. Thorsteinsdottir<sup>1</sup>, S. Jonsson<sup>2</sup>, H.H. Jonsson<sup>1</sup>, A. Kong<sup>1</sup>, D.F. Gudbjartsson<sup>1</sup>, K. Kristjansson<sup>1</sup>, S. Arnason<sup>2</sup>, H.J. Isakson<sup>2</sup>, J. Hallgrimsson<sup>2</sup>, J.R. Gulcher<sup>1</sup>, K. Stefansson<sup>1</sup>.* 1) deCODE genetics, Reykjavik, Iceland; 2) Departments of Medicine and Pathology National University Hospital, Reykjavik, Iceland.

Lung carcinoma (LC) is the leading cause of death from cancer among males and females in many western countries. The dominant role of tobacco smoke as a causative factor in LC is well established while the effects of genetics are not. We investigated the contribution of genetic factors to the risk of developing LC by linking information on all 2756 LC patients diagnosed within the Icelandic population in the period of 1955-2002 to an extensive genealogical database containing all living Icelanders and most of their ancestors since the settlement of the country. This allowed us to estimate risk ratios (RR) for 1st, 2nd and 3rd degree relatives of LC patients. The RR for smoking was similarly estimated using a population of 10541 smokers who had smoked for over 10 years. We demonstrate here that a familial factor for LC extends beyond the nuclear family as evidenced by RRs significantly greater than 1 for 2nd(RR=1.40) and 3rd(RR=1.13) as well as 1st(RR=2.65) degree relatives of LC patients. This effect was strongest for relatives of early-onset LC patients (RR range 1.32-3.42). The hypothesis that this increased risk is solely due to the familiarity of smoking was rejected beyond the nuclear family with a single sided test on the RRs of LC versus the RR of smoking. These results underscore the importance of genetic predisposition in the development of LC, with its strongest effect in early-onset patients. The drop in RR between 1st, 2nd and 3rd degree relatives is consistent with multiple and/or interacting effects. However, shared environmental factors and/or assortative mating related to smoking are also important components as evident by a RR greater than one for spouses of LC patients.

Program Nr: 428 from 2003 ASHG Annual Meeting

**The Cancer Risk Assessment and Prevention Program Database enhances practical management and promotes clinical research of patient data within a cancer genetics program.** *J. S. Wilbur, M.K. McDonnell, B. Cady, R.D. Legare.* Program in Women's Oncology, Women & Infant's Hospital, Providence, RI.

With the increasing number of patients involved in cancer genetic programs, appropriate management of clinical as well as programmatic information is critical. For the accrual of data for clinical research, optimal patient care, effective risk reduction interventions and tracking program information, the use of database software becomes essential. A survey of cancer genetic specialists, via the National Society of Genetic Counselors cancer list-serve, identified that multiple institutions are using a variety of software products to manage cancer genetic clinic data. The Cancer Risk Assessment and Prevention Program (CRAPP) Database effectively organizes several levels of patient data into fields that can be easily defined using ACT! 6.0 database software. The CRAPP Database can be queried for research purposes in order to offer patients the most current testing, risk reducing and screening options. Specifically, follow-up questionnaires, patient satisfaction surveys and personalized reports allow for effective time management strategies in a growing clinic. The database also contains multiple template letters and reports designed to retrieve data field information for the verification of ancillary revenue sources. The CRAPP Database currently maintains up to 600 patients per site, operates in three separate institutions and is network enabled to accommodate cancer genetic outreach clinics. When used via a network, data entries made by multiple users at different sites simultaneously update a single database. To comply with HIPPA guidelines, the CRAPP Database is accessible only by an authorized username and password. This method of data management decreases the need for administrative staff thereby diminishing program expenses. In addition to providing structure and organization to cancer genetic programs, the CRAPP Database is an effective means for the management of medical information creating a powerful resource for clinical and translational research as well as documenting programmatic data vital for the continual growth of cancer genetic services.

**Do parents and grands-parents of peoples with achondroplasia have a higher cancer risk?** *C. Stoll*<sup>1</sup>, *J. Feingold*<sup>2</sup>.  
1) Service de Gntique Mdicale Strasbourg, France; 2) University Paris 7.

Several studies, performed according to hypotheses based on teratogenesis and cancerogenesis have tried to answer the question : do parents of children with congenital anomalies have a higher cancer risk ? If the general answer is no, however a higher risk for cancer was reported in the parents of children with cleft/lip palate. In achondroplasia the neo-mutations are from paternal origin raising the hypothesis of the existence of a mutator gene which may favored also the occurrence of cancer. A questionnaire was sent to members with non familial achondroplasia of two associations of little people, one french, and one from Quebec, we asked for cancer, lymphoma and leukemia in their parents and grandparents. In the hypothesis tested the maternal lineage was the control. Hundred forty eight answers were obtained from 76 males and 72 females with achondroplasia. Out of them 68 have parents and/or grandparents with cancer. Eight fathers and 2 mothers of patients with achondroplasia had cancer. Among the grandparents of achondroplastic dwarfs there were 36 cancers including 2 lymphomas in the paternal grandfathers, 20 cancers including 2 chronic myeloid leukemia (CML) in the paternal grandmothers, 22 cancers including 2 CML in the maternal grandfathers, and 4 cancers in the maternal grandmothers. Paternal grandfathers and grandmothers had significantly more cancers than maternal grandfathers and grandmothers ( $X=4.43$ ,  $p<0.05$ ). In conclusion this study showed that there were significantly more cancers in the paternal grandparents of patients with achondroplasia than in their maternal grandparents, the controls raising the question of the occurrence of a mutator phenotype not restricted to cancer cells, but also involving germ cells and being passed to the descendents.

**Clinical and Molecular Genetic Studies of Bilateral Adrenal Hyperplasias: Genomic Scale cDNA Microarrays Profiling, Molecular Analysis of the cyclic AMP-Dependent Protein Kinase Regulatory Subunit 1A (PRKAR1A) Gene and Evidence Suggested Multistep Adrenocortical Tumorigenesis.** I. Bourdeau<sup>1,2</sup>, D. Lorang<sup>3</sup>, S.K. Libutti<sup>3</sup>, A. Lacroix<sup>2</sup>, C.A. Stratakis<sup>1</sup>. 1) SEGEN/DEB/NICHD, NIH, Bethesda, MD; 2) Endocrinology, Hotel-Dieu, CHUM, Montreal, QC, Canada; 3) Surgery Branch, NCI, Bethesda, MD.

Molecular genetic alterations in most adrenal tumors remain unknown. Gs and regulatory subunit 1 alpha of the protein kinase A (*PRKARIA*) mutations may lead to bilateral adrenal hyperplasias and to unilateral adenomas. Genetic analysis of primary adrenocortical hyperplasia may be informative for the identification of events leading to adrenocortical tumor formation. We studied 20 patients with ACTH-independent macronodular adrenal hyperplasia (AIMAH) (n=8) and primary pigmented nodular adrenal disease (PPNAD)(n=12). Screening for *PRKARIA* mutation was negative in the AIMAH group however 8 out of 12 PPNAD patients were mutated. Using cDNA microarray we compared hyperplastic tissues to a pooled of 62 normal adrenal glands then the gene profile of the AIMAH and PPNAD groups were compared. Total RNAs were fluorescently labeled and hybridized on glass slides containing 10 000 cDNA clones. The PPNAD group included a woman carrying a new *PRKARIA* mutation [Exon7 IVS del(-5-107)] who underwent bilateral adrenalectomy. Both hyperplastic adrenals showed the wild type and the deleted alleles whereas only the deleted allele was found in a single adenoma rising from the right hyperplastic gland. Microarray analysis revealed several genes consistently expressed in both groups. Genes involved in adrenal steroidogenesis including *CYP11A* (Cholesterol side-chain cleavage) were downregulated. The common upregulated genes including ATP-binding cassette, DEAD/H box polypeptide 5 (*DDX5*), activator of S phase kinase (*ASK*) and catenin (cadherin-associated protein)-like 1 (*CTNNAL1*). This data is a novel description suggesting that multistep adrenocortical tumorigenesis may occur in PPNAD. The gene expression profile of both adrenal hyperplasia may provide clues for investigation of molecular pathways involved in adrenal tumorigenesis.

**Modification of *BRCA1*-Associated Breast Cancer Risk by Smoking and Metabolism Genes.** *S. Colilla*<sup>1</sup>, *M.A. Blackwood-Chirchir*<sup>1</sup>, *H.T. Lynch*<sup>2</sup>, *S.A. Narod*<sup>2</sup>, *J.E. Garber*<sup>2</sup>, *T. Wagner*<sup>2</sup>, *M.B. Daly*<sup>2</sup>, *O.I. Olopade*<sup>2</sup>, *C. Isaacs*<sup>2</sup>, *S. Neuhausen*<sup>2</sup>, *G. Tomlinson*<sup>2</sup>, *H. Anton-Culver*<sup>2</sup>, *S.E. Plon*<sup>2</sup>, *J. Schildkraut*<sup>2</sup>, *B.L. Weber*<sup>1</sup>, *T.R. Rebbeck*<sup>1</sup>. 1) University of Pennsylvania, Philadelphia, PA; 2) The MAGIC Consortium.

Cigarette smoke may influence breast cancer risk by mutagenesis or by modulating endogenous hormones. To explore the relationship of smoking and breast cancer in women with *BRCA1* mutations, we evaluated breast cancer risk modifying effects of smoking with *NAT2* or *AIB1* genotype. 1115 women with *BRCA1* mutations were evaluated for *NAT2* and *AIB1* genotypes and pre-diagnosis smoking habits. Cox proportional hazards models, accounting for birth year and reproductive history, was used to model the risk of developing breast cancer. Ever smoking (HR=0.99, 95% CI: 0.80,1.24) and 30 pack years of smoking (HR=1.08, 95%CI: 0.85-1.37) were not associated with breast cancer risk. However, breast cancer risk was reduced in women who smoked 30 pack years (HR=0.47, 95%CI: 0.23-0.95) compared to nonsmokers. Later age of smoking initiation was also associated with decreased breast cancer risk (HR=0.94; 95% CI: 0.91-0.98). Slow *NAT2* acetylators had increased breast cancer risk (HR=1.34, 95% CI: 1.0-1.78) but *NAT2* did not modify the smoking-breast cancer relationship. As shown previously, women with 29 *AIB1*-CAG repeats were at significantly increased breast cancer risk (HR=1.70, 95% CI: 1.16-2.49). Never smokers with 29 *AIB1*-CAG repeats were at highest breast cancer risk (HR=1.79, 95% CI: 1.07-3.00). Heavy smokers with 29 *AIB1*-CAG repeats and 30 pack years were at lowest breast cancer risk (HR=0.19, 95% CI: 0.07-0.48). No increased risk was conferred to heavy smokers with 29 *AIB1*-CAG repeats [HR=0.77, 95%CI: 0.28-2.11]. Therefore, *BRCA1*-associated breast cancer risk may depend in part on *AIB1* genotype and smoking. Since *AIB1* mediates endogenous hormone exposures and smoking depresses circulating hormone levels, these results present a biologically plausible pathway of hormone-mediated breast carcinogenesis in *BRCA1* mutation carriers.

**The relationship of allelic imbalance(AI)patterns in a MSS young onset(<50 year of age)colorectal cancer(CRC) compared to older onset(>65 years of age)CRC: predominance of rectal cancer in young onset CRC. L.**

*Boardman, R. Johnson, J. Slusser, M. de Andrade, B. Morlan, S. Schmidt, G. Petersen, S. Thibodeau.* Mayo Clinic, Rochester, MN.

**Introduction:**In the US, 15% of the CRC burden occurs in people <50 years of age. The cause for the majority of young onset CRC remains unknown. Discovery of genes responsible for CRC using AI has focused on sporadic, older onset CRC. **Methods:** Study subjects were selected from an IRB-approved protocol, Colorectal Risk Assessment (CRA), a prospective tumor collection of all CRC surgically treated at our institution from 1994-1997. 45 consented cases of MSS young onset CRC, excluding HNPCC or FAP, were identified. Ninety MSS older onset CRC with CRA consent were randomly selected on the sole criterion of age to serve as controls. We evaluated both the young and older onset CRC tumors for AI using 96 genome wide dinucleotide repeat markers from the ABI Prism Linkage Mapping set (Perkin-Elmer Corp., Foster City, CA). AI was assessed as positive if the calculated difference between the normal and tumor alleles was greater than or equal to 2. **Results:** Young onset patients were approximately three times more likely to present with rectal cancer than older onset patients ( $p=0.0015$ ). Of the young onset CRC cases, 51% (23/45) occurred in the rectum and 49% (22/45) developed in the colon proximal to the splenic flexure. Consistent with previous studies, AI on 17p, 18q, 5q, 20p, 21q and 15q occurred in at least one quarter of the study subjects. Within the young onset group, AI was detected on 6q in 18 to 21% of rectal and colon tumors, respectively, but in <1% of both older onset groups. AI was found in 30% of the older onset rectal tumors, 20% of young onset rectal cancer, and 13% of young and older onset colon tumors. **Conclusion:** MSS young onset CRC may arise more frequently in the rectum in young onset patients. Larger studies are required to confirm this age-related site difference. AI patterns in MSS young and older onset CRC indicate that further larger scale studies categorizing AI by age and site may be an efficient means to define genetic events unique to young onset CRC.

**The prevalence of Human Papilloma Virus Infection in cervical cancer patients from Reference Center-Iran.** *N. Kohannia*<sup>1,4</sup>, *E. Keyhani*<sup>1</sup>, *A. Hatami*<sup>1</sup>, *R. Amini*<sup>2</sup>, *N. Izadi*<sup>3</sup>, *L. Abbasi Moheb*<sup>1</sup>, *H. Najmabadi*<sup>1</sup>. 1) Genetics Research Center, USWR university, Tehran, Tehran, Iran; 2) Reference Laboratory of Iran Research Center- Tehran, Iran; 3) Tehran University-Mirzakouchakkhan Hospital- Tehran, Iran; 4) Islamic Azad University- Lahijan, Iran.

Human papiloma virus (HPV) is a well-known etiologic factor of cervical cancer in Western countries. However, despite a relatively high prevalence of cervical cancer in Iran, no studies are available on the relationship between HPV infection and cervical cancer. A retrospective study was performed on 110 cervical cancer paraffin-embedded histopathologic blocks. DNA extraction was performed through standard procedures followed by Multiplex Polymerase Chain Reaction amplification with two pairs of primers (one as internal control) which were designed to detect HPV in the related tissues. The products were run on 8% PAGE. HPV typing was done for the types 16 and 18 positive controls of which were available in Iran. In our study more than 70% of the malignant cervical lesions were contaminated with HPV. This finding confirms the previous reports on the significant association of HPV with cervical cancer.

**Analysis of paraffin embedded human breast cancer cell line xenografts by IS-RT-PCR reveals in vitro and in vivo differences MMP.** *L.M. Haupt<sup>1</sup>, E.W. Thompson<sup>2</sup>, A.E.O. Trezise<sup>3</sup>, R.E. Irving<sup>1</sup>, M.G. Irving<sup>4</sup>, L.R. Griffiths<sup>1</sup>.* 1) Genomics Research Center, School of Health Science, Griffith University Gold Coast, Southport, Queensland, Australia 4215; 2) VBCRC Invasion and Metastasis Unit, St. Vincent's Institute of Medical Research and University of Melbourne, Department of Surgery, Melbourne, Victoria, Australia 3065; 3) School of Biomedical Sciences, University of Queensland, St Lucia, Queensland 4072; 4) Institute of Health Sciences, Bond University, Queensland, Australia 4229.

**Purpose:** We applied the in situ-reverse transcription-polymerase chain reaction (IS-RT-PCR) method to examine MT1-MMP (MMP-14, membrane type-1-MMP), MMP-1 (interstitial collagenase) and MMP-3 (stromelysin-1) gene expression in human breast cancer (HBC) cell lines in vitro and in vivo. **Methods:** Combining the techniques of ISH and RT-PCR, IS-RT-PCR allows the detection and localisation of discrete low abundance mRNA sequences in situ. In vitro, MMP gene expression was examined following exposure to Con A. In vivo, we examined differences between the MMP-inductive effects using archival paraffin embedded xenografts derived from various HBC cell lines (MDA-MB-231, MDA-MB-435, MCF-7 and Hs578T). **Results:** In vitro, exposure to Con A caused increased MT1-MMP gene expression in MDA-MB-231 cells and decreased MT1-MMP gene expression in MCF-7 cells. MMP-1 and MMP-3 gene expression levels remained unchanged in both cell lines. In vivo, the stromal cells recruited into each xenograft demonstrated important differences. Estrogen treatment stimulated gene expression of MT1-MMP in the stromal cells around MCF-7 cells. No MMP gene expression was detected in the stroma of the MDA-MB-231 spleen metastasis or the MDA-MB-435 stroma. Hs578T cells elicited a strong stromal gene expression of MMP-3 compared to MT1-MMP and MMP-1. **Conclusion:** These results demonstrate potentially important differences in MMP expression between in vitro and in vivo model systems, and differential stromal induction in the xenograft setting. In addition, the data highlight the contribution of the stroma to MMP gene expression and the complexity of stromal-epithelial interactions within breast carcinoma.

**Prostate Cancer Cell Line PC-3 Lacks Expression Of The Cell Adhesion Molecule CEACAM1.** *O.A. Schirripa<sup>1</sup>, J.N. Weitzel<sup>1</sup>, J.E. Shively<sup>2</sup>.* 1) Clinical Cancer Genetics, City of Hope National Medical , Duarte, CA; 2) Division of Immunology, Beckman Research Institute of the City of Hope, Duarte, CA.

Background: Cell-cell and cell-matrix interactions help to maintain a differentiated cell in a structurally and functionally balanced state. Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), which is a subfamily in the immunoglobulin superfamily, is a cell-cell adhesion molecule with tumor-suppressive activity that has major influences on epithelial cell development, differentiation, and apoptosis. CEACAM1-4S, an isoform molecule of CEACAM-1 was found to mediate apoptosis and revert human mammary carcinoma cells to a normal morphogenic phenotype in a 3D Matrigel culture. It is our hypothesis that the molecular mechanisms that govern cell-cell and cell-matrix interactions in the mammary gland also are involved in the development and progression of prostate cancer. Using monoclonal antibodies, CEACAM1 has been shown to be down regulated in higher Gleason grades of prostate cancer. Method: Western blot analysis was performed on lysates of PC-3 cells for CEA, CEACAM1 and CEACAM6 using antibodies to the N extracellular domain (T84.1, monoclonal ), long cytoplasmic tail (22.9, polyclonal) and CEACAM1 extracellular domain (4D1C2, monoclonal). Monoclonal antibody 5F4 specific for CEACAM1 was used on PC-3 cells grown in Matrigel for 12 days Results: PC-3 cells are negative for CEACAM1 and CEACAM6 and CEA positive by Western blot. PC-3 cells grown in Matrigel for twelve days formed spheroids and were negative for CEACAM1 by immunohistochemistry. Conclusion: We report the absence of CEACAM1 expression on the PC-3 prostate cancer cell line. PC-3 cells grown as 3D spheroids in Matrigel may provide a model system to further elucidate the structure and function of specific CEACAM1 isoform expression patterns in human prostate cancer cells. Transfection experiments of PC-3 cells with different isoforms of CEACAM1 are currently underway to see if spheroids will revert to a normal morphology and form lumina in Matrigel. Supported in part by the NCI - Grant # 2R25 CA75131 and the California Cancer Research Program of the University of California - Grant # 99-86874.

**Association of ELAC2, MSR1, and RNASEL in the etiology and severity of prostate cancer.** *H. Rennert, K. Addya, C.M. Zeigler-Johnson, E.E. Spangler, A.H. Walker, S. Restine, S.B. Malkowicz, D.G.B. Leonard, T.R. Rebbeck.*  
University of Pennsylvania, Philadelphia, PA.

Family studies have identified three prostate cancer (PC) susceptibility genes, ELAC2/HPC2 (17p), RNASEL/HPC1 (1q25) and MSR1 (8p22). We evaluated the role of these genes in PC risk in a mixed ethnicity U.S. population. The frequency and association with PC of 14 putative susceptibility alleles in these three genes were studied in 827 incident PC cases and 714 controls. 26% of study participants were African American. Assays were performed using the ABI PRISM 7900HT Sequence Detection System. The ELAC2 E622V allele was observed only in Caucasians with a frequency of <0.3%. At MSR1, 4.4% of individuals carried S41Y, V113A, D174Y, R293X, or H441R. All variants studied were seen in Caucasians, while only V113A, D174Y, and H441R were seen in African Americans. No statistically significant effects were observed for rare MSR1 variants and PC risk, but these variants conferred increased risk of extracapsular extension (age- and race-adjusted odds ratio (OR)=2.7, 95%CI: 1.2-6.3). At RNASEL, 1.3% of Caucasians and no African Americans carried E265X or 354C>T. R462Q was carried by approximately 30% of individuals regardless of race. Individuals who inherited one or more of the RNASEL variants were at increased risk of PC (OR=3.5, 95%CI: 1.0-12.5), and more likely to have a family history of PC (OR=1.5, 95%CI=1.0-2.1), an earlier age at PC diagnosis ( $p=0.04$ ) and extracapsular extension (OR=4.0, 95%CI: 1.1-15.4). Carriage of rare variants at either RNASEL (E265X or 354C>T) or MSR1 (S41Y, V113A, D174Y, R293X, or H441R) was associated with an increased PC risk (OR=2.3, 95% CI: 1.1-4.7) and elevated probability of extracapsular extension (OR=2.8, 95% CI: 1.1-6.9). These results suggest that RNASEL and MSR1 are associated with PC etiology and tumor severity.

**Use of functional prediction models to evaluate the association of BRCA1 and 2 variants to breast cancer risk factors.** *D. Peel, A. Ziogas, H. Hoang, H. Anton-Culver.* Epidemiology Div, Univ of Calif, Irvine, Irvine, CA.

Limited data are available regarding the role of common variants in BRCA1 & 2 in breast cancer risk. We examined the relationship of variants in BRCA1 & 2 genes to early-onset (< age 35) breast cancer risk. To assign a level of significance to the variant proteins, we used 4 established Protein Structure and Activity Prediction Models (PSAPM): SIFT algorithm (emphasizes evolutionary conservation of protein sequence), PolyPhen algorithm (emphasizes aspects of the protein structure), % conservation (determines the level of conservation across species), and SSPRO (emphasizes secondary protein structure). We sequenced BRCA1 & 2 in 137 early-onset, population-based breast cancer cases to identify mutations and variants. For analysis of variants we included all 120 mutation negative cases. Using the 4 PSAPM, we determined scores for each of the variants for both the BRCA1 & 2 genes. We ranked the scores and calculated sum-scores across the variants for each sample. Statistical evaluation was performed to test possible associations between these scores and known breast cancer risk factors (family history of breast and ovarian cancer, reproductive history, and tumor characteristics) in the population under study. Although each of the PSAPM scores is independently assessed and partly based on different aspects of protein function/structure for each variant, significant correlation and agreement among the scores of the 4 PSAPM were found, indicating that the prediction potential of any one of these models can be used reliably in the identification of informative variants. Significantly higher PSAPM scores of BRCA1 but not BRCA2 were observed in nulliparous women compared to parous women. Positive family history of breast and ovarian cancer was significantly associated with high PSAPM scores in BRCA1 but not BRCA2. Significantly higher PSAPM scores of variants in both BRCA1 & 2 were associated with aggressive tumor behavior characteristics (tumor size and ER/PR negative tumors). This study demonstrates the use of PSAPM and indicates that non-mutation variants in the BRCA1 & 2 genes have a role in the characterization and evaluation of breast cancer risk.

**Comparative genomics of an evolutionary breakpoint region regularly deleted in human tumors.** *E.A. Darai, M. Kost-Alimova, H. Kiss, L. Fedorova, H. Kansoul, I. Kholodnyuk, G. Klein, S. Imreh.* Microbiology and Tumorbiology Center, Karolinska Institute, Stockholm, Sweden.

We developed a functional assay called the Elimination Test (Et) for identification and fine mapping of physically or functionally eliminated chromosomal regions containing tumor antagonizing genes. Et is based on the introduction of a normal human chromosome into murine or human tumor cells by microcell fusion. The resulting microcell hybrids are serially passaged through SCID mice, and the derived tumors are analyzed for regular eliminations of chromosomal segments by molecular and cytogenetic methods. The Et was focused on the short arm of chromosome 3 due to its frequent deletion in a large number of tumor types. A putative tumor suppressor region, CER1 at 3p21.3 was identified in the tumor panel derived from SCID mice. Large-scale comparison of genomic sequences between species allows characterizing important gene- or locus-specific regulatory elements. For characterization of our CER1 genes we compared 1.32 Mb of CER1 from human chromosome 3p21.3 to its orthologous regions on mouse chromosome 9F. The corresponding mouse region was found divided into two blocks, but their gene content and gene positions were highly conserved. We also recognized a large number of conserved elements that were neither exons, CpG islands nor repeats. We identified and characterized 5 novel orthologous mouse genes. Human CER1 belongs to two conserved chromosomal segments in mouse, containing a murine/human conservation breakpoint region. Several breaks occurred in tumors within the region surrounding CBR and this sequence has features that characterize unstable chromosomal regions: deletions in YAC clones, late replication, gene and segment duplications, pseudogene insertions. We extended our analysis of CER1 to other species: *C. elegans*, *Drosophila melanogaster*, *Fugu rubripes*. It was found that in all these organisms the genes from the telomeric part of CER1 are evolutionary conserved. The part of CER1 surrounding CBR contains genes, which are modified through evolution showing lower homology, duplications and changes of function. An evolutionary new gene TMEM7 was found in this region.

**Marsupial BRCA1: Conserved regions in mammals and the potential effect of missense changes.** *E.A. Ostrander<sup>1</sup>, C.J. Ramirez<sup>1</sup>, J.D. Potter<sup>2</sup>, M.A. Fleming<sup>1</sup>, G.K. Ostrander<sup>3</sup>*. 1) Clinical Div, Fred Hutchinson Cancer Res Ctr, Seattle, WA; 2) Division of Public Health Sciences, Fred Hutchinson Cancer Res Ctr, Seattle WA; 3) Departments of Biology and Comparative Medicine, Johns Hopkins University, Baltimore, MD.

More than half of reported missense changes in the breast cancer susceptibility protein BRCA1 occur in exon 11, but none has been clearly identified as disease-associated and only 28 are designated "probable" neutral polymorphisms. Previously, in a comparison of sequences from 57 eutherian mammal species, we found seven "highly conserved regions" between amino acids 282 and 1103 and identified 38 missense changes as likely to disrupt gene function. These conserved regions were also present in birds and amphibians and included only six of the mutations predicted to affect function. In this new analysis, we hypothesize that using 37 ancestral sequences derived from the 57 GenBank sequences and including three of eight newly cloned marsupial sequences would allow us to identify regions unique to mammals and refine our predictions of disease-associated missense changes. We identified 13 conserved regions, three of which appear to be unique to mammals, and 21 likely disease-associated missense changes, 11 of which occur in conserved regions. Seven regions identified in this analysis, including the three found only in mammalian sequences, and nine missense changes predicted to affect function are in the putative STAT1 interaction domain suggesting that the roll of STAT1 in immune response is important to mammary function. The reduction in the number of missense changes predicted to be disease-associated and the identification of conserved regions specific to mammals can facilitate the further study of the role of missense changes in BRCA1 associated breast cancers.

**Genome-wide linkage scan reveals evidence for hereditary prostate cancer gene on chromosome 17q. *E.M.***

*Lange*<sup>1</sup>, *E.M. Gillanders*<sup>2</sup>, *C.C. Davis*<sup>1</sup>, *W.M. Brown*<sup>1</sup>, *J.M. Trent*<sup>2</sup>, *J.E. Montie*<sup>3</sup>, *K.A. Cooney*<sup>3</sup>. 1) Public Health Sci, Wake Forest Univ Sch Medicine, Winston-Salem, NC; 2) National Human Genome Res Inst, Bethesda, MD; 3) Internal Med and Urology, Univ of Michigan Sch Medicine, Ann Arbor, MI.

Previous linkage studies have suggested prostate cancer susceptibility genes located on chromosomes 1, 20, and X. Several putative prostate cancer candidate genes have also been identified including RNASEL, MSR1, and ELAC2/HPC2. Presently, these linkage regions and candidate genes appear to explain only a small proportion of hereditary prostate cancer cases suggesting the need for additional whole genome analyses. We conducted a genome-wide mode-of-inheritance-free linkage scan, using 405 genetic markers on 176 pedigrees from the University of Michigan Prostate Cancer Genetics Project (PCGP). Stratified linkage analyses were completed using previously established criteria. Results based on the entire set of 176 pedigrees showed strong suggestive evidence for linkage on chromosome 17q (LOD = 2.36), with the most significant evidence coming from the subset of pedigrees with four or more affected individuals (LOD = 3.27). Interestingly, these linkage peaks were centered directly over the BRCA1 gene. Race-specific analyses also revealed strong suggestive evidence for linkage in our African American pedigrees on chromosome 22q (LOD = 2.35). Given the linkage data, we are currently sequencing the BRCA1 gene from affected family members in chromosome 17q-linked families to better our understanding of the potential role of BRCA1 in hereditary prostate cancer.

**Recruitment of high-risk prostate cancer families in Southern Louisiana: challenges and successful strategies.**

*D.M. Mandal<sup>1</sup>, T.N. Turley-Stoulig<sup>1</sup>, S.L. Halton<sup>1</sup>, E.M. Gillanders<sup>2</sup>, J. Carpten<sup>2</sup>, J. Trent<sup>3</sup>, J.E. Bailey-Wilson<sup>2</sup>, W. Rayford<sup>4</sup>.* 1) Dept. of Genetics, LSU Health Sciences Ctr, New Orleans, LA; 2) NHGRI/NIH, Bethesda, MD; 3) Translational Genomics Research Institute, Phoenix, AZ; 4) Dept. of Urology, LSU Health Sciences Center, New Orleans, LA.

There is a trend of increasing risk in prostate cancer with increasing number of affected relatives and earlier age of onset in affected relatives. To date, three genes have been successfully cloned. However, more data are needed to confirm the suggested linkages and to identify and characterize specific mutations in diverse populations. We have initiated a study to ascertain families with history of 3 prostate cancer cases through the prostate cancer screening program at the LSUHSC Department of Urology, from participating local urologists and collaborative hospitals from Southern Louisiana. Entering participants into the study has been very challenging due to the raised concern of patient confidentiality and privacy issues but we have been able to build some successful collaboration through innovative approaches. Thus far, 224 individuals have been interviewed. Of those, nine reported a significant family history of prostate cancer. Biological sample collection and pathological confirmation are complete on three of those families, which are in the process of genome screening. Follow up is ongoing in the remaining six families. Description of the data resource will be presented and the critical issues involved in recruitment in a genetic study will be discussed, with particular emphasis on the challenges and innovative strategies in recruitment of families in a southern rural setting.

**Genome-wide search for novel breast cancer susceptibility genes in Australian multiple case kindreds.** *G.M. Pupo*<sup>1</sup>, *B. Newman*<sup>2</sup>, *D.J. Venter*<sup>3</sup>, *J.L. Hopper*<sup>4</sup>, *G. Chenevix-Trench*<sup>5</sup>, *kConFab*<sup>6</sup>, *G.J. Mann*<sup>1</sup>. 1) Westmead Institute for Cancer Research, University of Sydney at the Westmead Millennium Institute, Westmead, NSW 2145, Australia; 2) School Of Public Health, Queensland University of Technology, Kelvin Grove, QLD 4059 Australia; 3) Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, VIC 3042, Australia; 4) Centre for Genetic Epidemiology, University of Melbourne, Carlton, VIC 3053, Australia; 5) Queensland Institute for Medical Research, Royal Brisbane Hospital, Herston, QLD 4029, Australia; 6) Kathleen Cuninghame Foundation Consortium for Research into Familial Breast Cancer.

Mutations in the known breast cancer susceptibility genes BRCA1, BRCA2 and ATM account for only part of the familial clustering of this disease and there is good evidence that further major susceptibility (BRCAX) loci remain to be found. We have been using the resources of the Kathleen Cuninghame Foundation Consortium for Research into Familial Breast Cancer (kConFab) and those of the Australian Breast Cancer Family Study (ABCFS) to identify kindreds suitable for mapping BRCAX loci. These were selected on the basis of having at least three genotypable individuals with female breast cancer, no cases of male breast cancer, and with few exceptions no cases of ovarian cancer. Clinic-based BRCA1 and BRCA2 mutation testing was negative, except for common population polymorphisms, and the probability of occult involvement of the BRCA1 and BRCA2 genes was reduced by exclusion of kindreds in which a haplotype of either BRCA1- or BRCA2-flanking markers was shared by all affected individuals. A 10cM genome-wide search was carried out in 40 such kindreds using the 400 marker ABI v2 panel. The highest LOD score under heterogeneity (HLOD) was 2.16 (non-parametric LOD 1.83,  $p=0.04$ ); several other regions with HLODs = 1.5-2.0 also merited investigation by further markers and/or fine mapping which is currently under way. Subsets based on age of onset and presence of other cancers correlated to some extent with particular linkage peaks, and this will also be discussed.

**Dietary factors and occurrence of microsatellite instability in sporadic colon carcinomas.** *B. Diergaarde*<sup>1,2</sup>, *H. Braam*<sup>3</sup>, *G. Van Muijen*<sup>3</sup>, *M. Ligtenberg*<sup>3</sup>, *F. Kok*<sup>2</sup>, *E. Kampman*<sup>2</sup>. 1) Public Health Sciences, Fred Hutchinson Cancer Res. C., Seattle, WA; 2) Human Nutrition & Epidemiology, Wageningen University, Wageningen, The Netherlands; 3) Pathology, UMC St Radboud, Nijmegen, The Netherlands.

Microsatellite instability (MSI) occurs in 10-20% of the sporadic colon carcinomas and appears to be primarily due to alterations in the mismatch repair genes *hMLH1* and *hMSH2*. Little is known about the role of diet in MSI-related colon carcinogenesis. We used data from a Dutch population-based case-control study (184 cases; 259 controls) on sporadic colon carcinomas to evaluate associations between dietary factors and occurrence of MSI, *hMLH1* expression and *hMLH1* promoter hypermethylation. Usual dietary habits were assessed by an interview-based questionnaire. Case-case and case-control comparisons were conducted. Odds ratios (OR) and the corresponding 95% confidence intervals (95% CI) were calculated using multiple logistic regression models. Red meat intake was significantly different related to MSI-H tumors than to MSI-L/MSS tumors (MSI-H vs. MSI-L/MSS, OR: 0.3, 95% CI: 0.1-0.9). A positive association was observed with MSI-L/MSS tumors (OR: 1.5, 95% CI: 0.9-2.6) when compared with the population-based controls, an inverse association with MSI-H tumors (OR: 0.5, 95% CI: 0.2-1.2). Fruit consumption was significantly different related to MSI-H tumors with hypermethylated *hMLH1* (Methyl<sup>+</sup> tumors) than to MSI-H tumors without hypermethylated *hMLH1* (Methyl<sup>-</sup> tumors) (Methyl<sup>+</sup> vs. Methyl<sup>-</sup>, OR: 0.2, 95% CI: 0.1-0.9; Methyl<sup>+</sup> vs. controls, OR: 0.4, 95% CI: 0.2-0.9; Methyl<sup>-</sup> vs. controls, OR: 1.2, 95% CI: 0.8-1.7). Vegetable consumption lowered the risk of MSI-H and MSI-L/MSS tumors but like most other evaluated dietary factors was not distinctively associated with a specific MSI or *hMLH1* promoter methylation status. Associations observed with tumors that showed absence of *hMLH1* expression (n=26) generally did not differ significantly from those observed with MSI-H tumors. Our data suggest that red meat consumption promotes the development of MSI-L/MSS carcinomas in particular, whereas fruit intake seems to especially decrease the risk of MSI-H carcinomas exhibiting epigenetically silenced *hMLH1*.

***Helicobacter pylori* infection in gastric carcinogenesis.** *S. Myllykangas*<sup>1</sup>, *O. Monni*<sup>3</sup>, *H. Rautelin*<sup>2</sup>, *S. Knuutila*<sup>1</sup>. 1) Department of Medical Genetics, Haartman Institute, University of Helsinki and Helsinki University Central Hospital Laboratory Diagnostics, Helsinki, Finland; 2) Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki and Helsinki University Central Hospital Laboratory Diagnostics, Helsinki, Finland; 3) Biomedicum Biochip Center, University of Helsinki, Helsinki, Finland.

We studied human gene expression changes caused by *Helicobacter pylori* infection using an in vitro model and 13k cDNA microarrays. An AGS cell line was infected with *H. pylori* strain NCTC 11637. *H. pylori* infection was found to induce differential expression of genes located in chromosomal locations known to contain frequent chromosomal aberrations and gene mutations specific to gastric cancer. 17q11-q25 location comprised the most *H. pylori* infection responsive genes. 17q11-q25 is one of the most frequently amplified chromosomal locations associated with gastric cancer. Based on our time series experiments, the primary transcription target of the infection seemed to be *FOS*, the expression of which significantly increased quickly after *H. pylori* infection. Also *FOSL1* was overexpressed immediately after infection. *c-fos* and *Fra-1*, encoded by *FOS* and *FOSL1* genes, are part of the AP-1 transcription factor complex. *H. pylori* infection also activated transcription of several stress response genes. Genes encoding heat shock proteins HSP40 and HSP70 as well as genes activated in DNA damage and growth arrest, such as *GADD45A*, were upregulated. Our results suggest that *H. pylori* infection induced stress response might be connected with the emergence of DNA damage in the host genome. The open and active chromatin responding to *H. pylori* infection might be sensitive to injury, as it is not protected by the histones. This could explain the gene and structure aberrations frequently observed in the chromosomal locations specific to gastric cancer.

**Prospective risk of pancreatic cancer in familial pancreatic cancer kindreds.** *A.P. Klein<sup>1</sup>, K.A. Brune<sup>2</sup>, M. Goggins<sup>2,3,4</sup>, A.C. Tersmette<sup>5</sup>, J.A. Offerhaus<sup>5</sup>, G.M. Petersen<sup>6</sup>, S.E. Kern<sup>2,3</sup>, R.H. Hruban<sup>2,3</sup>.* 1) Statistical Genetics Section, NIH/NHGRI/IDRB, Baltimore; 2) Dept. of Pathology, Johns Hopkins School of Medicine, Baltimore; 3) Dept. of Oncology, Johns Hopkins School of Medicine, Baltimore; 4) Dept. of Medicine, Johns Hopkins School of Medicine, Baltimore; 5) Dept. of Pathology, Academic Medical Center, Amsterdam, The Netherlands; 6) Mayo Clinic, Rochester, MN.

**Background.** Pancreatic cancer is the 4th leading cause of cancer death in the United States, leading to an estimated 30,000 deaths in 2003. Numerous studies show that a family history of pancreatic cancer is a significant risk factor. The prospective risk of developing pancreatic cancer among those with a strong family history of the disease has not been well studied. The aim of this study was to quantify the prospective risk of developing pancreatic cancer among individuals with a family history of this disease. **Methods.** In a registry-based study, we estimated the risk of pancreatic cancer in individuals with a family history of pancreatic cancer. Person-years of follow-up were calculated for each individual from their time of enrollment in the registry until the date health information was last updated, date of pancreatic cancer diagnosis or death. Standardized incidence ratios (SIR) were calculated by comparing the observed number of incident pancreatic cancer cases among individuals enrolled in the National Familial Pancreas Tumor Registry (NFPTR) to those expected under SEER incidence rates. **Results.** 5,199 individuals over 1,000 kindreds were followed for a total of 13,910 person-years. 22 incident pancreatic cancers occurred in NFPTR kindreds, 19 of which were eligible to be included in this study. Risk was elevated in individuals with three (39.2, 95% C.I.=14.4-85.4), two (6.2, 95% C.I.=1.7-15.8), or one (2.7, 95% C.I.=0.7-6.9) first-degree relatives with pancreatic cancer. **Conclusions.** These results: a) demonstrate the existence of familial pancreatic cancer; b) provide a rational basis for cancer risk counseling; and c) emphasize the need for effective early-diagnosis screening techniques for at-risk family members.

**BRCA mutations and the risk of prostate and colorectal cancer in Ashkenazi Jews.** *T. Kirchhoff, N. Kauff, P. Kolachana, J. Satagopan, N. Mitra, H. Huang, J. Shia, C. Palmer, K. Nafa, N.A. Ellis, K. Offit.* Memorial Sloan-Kettering Cancer Center (MSKCC), New York, NY.

Germline mutations in the BRCA genes significantly increase the risk of developing breast or ovarian cancer. In the Ashkenazi Jewish (AJ) population, three founder mutations (185delAG and 5382insC in BRCA1 and 6174delT in BRCA2) account for >95% of the mutant alleles present in the population. Using a case-control design, we assessed the contribution of BRCA mutations to the risk of developing prostate cancer (PrC) or colorectal cancer (CRC). We genotyped 251 consecutive AJ PrC cases and 586 consecutive AJ CRC cases, ascertained at MSKCC from 2000-2003 and 1994-2002, respectively, for the three founder mutations using a modified restriction enzyme digestion analysis. Two groups of controls previously genotyped for the founder mutations were selected from volunteers participating in Washington Ashkenazi Study (WAS). For the PrC series, we selected all available PrC-free males (N=1472) and for CRC series we selected all available CRC-free persons (N=5012). A logistic regression model was used to estimate the relative risk for cancer due to BRCA mutations. We detected an elevated frequency of the three founder mutations in PrC cases (13/261=5.1%) versus controls (28/1472=1.9%). Of the three mutations, the BRCA2\*6174delT showed the largest increase (3.1% in cases vs. 1% in controls). After adjusting for age, we found the association was significant for PrC risk (OR=3.41; p=0.001; 95% CI: 1.64-7.06). However, there was not a significant increase in mutation carriers in PrC cases <50. An increased frequency of the founder mutations was not detected in CRC cases (6/586=1.02%) versus controls (118/5012=2.4%) and an increased risk was not observed (OR=0.50; p-value=0.10; 95% CI: 0.221.14). Our data suggest that BRCA mutations confer a three-fold increased risk of developing PrC but not of CRC. This case-control study design may be less biased than a family-based approach because the ascertainment was based on incident cancer cases.

**A frequent somatic mutation of the DOC-1 gene associated with microsatellite-unstable colorectal cancer. T.**

*Weber<sup>1</sup>, Z.Q. Yuan<sup>1</sup>, T.S. Kent<sup>1</sup>, Y. Wang<sup>2</sup>, A. Miller<sup>1</sup>, R. Hamelin<sup>3</sup>, W. Edelmann<sup>2</sup>, H.T. Lynch<sup>4</sup>.* 1) Surgery/Molec Gen, Ullmann1221, Albert Einstein Col Medicine, Bronx, NY; 2) Cell Biology, Albert Einstein College of Medicine, Bronx, NY; 3) INSERM, Paris, France; 4) Department of Preventive Medicine, Creighton University, Omaha, NE.

**Introduction:** p12DOC-1, a CDK2-associated protein, is a candidate cell growth suppressor. Previously, we demonstrated that absent or decreased expression of p12DOC-1 in microsatellite-unstable (MSI+) colorectal cancer (CRC) cell lines, or p12DOC-1 down-regulation, was associated with colorectal epithelial cell proliferation and decreased apoptosis. To further elucidate the relationship between decreased p12DOC-1 and MSI+ CRC, we screened the DOC-1 gene in MSI+ CRC samples. **Methods:** DNA from 50 CRC tissues and 21 CRC cell lines were obtained from several collaborating laboratories. Mismatch repair (MMR) status was assessed by MMR protein expression, MSI assay, and hypermutable assay. All samples were sequenced to look for mutations in the DOC-1 gene. p12DOC-1 mRNA and protein expression were assessed by real-time PCR and Western Blot assay. Cell proliferation and apoptosis status were determined by FACS assay. **Results:** A novel somatic mutation, delT at the 3' end of a poly (T)8 of the DOC-1 gene, was identified in 12% (5/41) of MSI+ CRC, but in none of the MSS samples (0/30). Interestingly, when wild-type MMR protein was induced in the in vitro recombinant system, the mutation was reversed and p12DOC-1 expression increased and colon epithelial cells proliferation was inhibited. **Discussion:** Because of the association between the DOC-1 mutation and MMR deficiency, our results strongly suggest that this mutation may be a pathologic mutation, leading to inactivation of DOC-1 and contributing to the development and progression of MSI+ CRC. Further investigation of the mutation status of DOC-1 in a larger number of MSI+ CRC samples are underway in our laboratory.

**Low expression of a novel gene TAF9L2, encoding a putative p53 binding domain, in neuroblastoma tumors.** C. Krona<sup>1</sup>, H. Carén<sup>1</sup>, F. Abel<sup>1</sup>, K. Ejeskär<sup>1,2</sup>, R.M. Sjöberg<sup>1</sup>, T. Martinsson<sup>1</sup>. 1) Dept. Clinical Genetics, Gothenburg Univ., Sahlgrenska Univ. Hosp. - East, S-41685 Gothenburg, Sweden; 2) Murdoch Childrens Research Institute, Melbourne, Australia.

Neuroblastoma is characterized by a lack of P53 mutations and no other tumor suppressor gene consistently inactivated has yet been identified in this childhood cancer form. Characterization of a new gene, denoted TAF9L2, in the neuroblastoma tumor suppressor candidate region in chromosome 1p36.22 reveals that TAF9L2 contains a predicted TFIID-31 domain, representing the TATA-binding-protein-associated factor, TAFII31, which is required for P53 mediated transcription activation. Two differential transcripts of this gene were shown to be ubiquitously expressed, one of them with an elevated expression in fetal tissues. Primary neuroblastoma tumors representing different stages of the disease showed either very weak or no measurable TAF9L2 expression, contrary to the level of expression observed in neuroblastoma cell lines. Furthermore, we determined the genomic organization of TAF9L2. Automated genomic DNA sequencing of the coding region of the gene as well as the promoter sequence in 42 neuroblastoma tumors did not reveal any loss-of-function mutations indicating that mutations in TAF9L2 is not a common abnormality of neuroblastoma tumors. We suggest that low expression of this gene, located in the neuroblastoma tumor suppressor candidate region in chromosome 1p, in embryonal cells impair the ability for apoptosis in defective cells through the P53-pathway.

**The Application of STS-QPCR for the High Resolution Physical Mapping of the Tumor Suppressor Locus *NRC1*.**

*K. Zhang*<sup>1</sup>, *L. Jin*<sup>1</sup>, *A.M. Killary*<sup>2</sup>. 1) Center for Genome Information, Department of Environmental Health, Univ Cincinnati, Cincinnati, OH 45267; 2) Department of Molecular Genetics, UT-MD Anderson Cancer Center, Houston, TX 77030.

A tumor suppressor locus *NRC1* (OMIM ID 604442) in renal cell carcinoma (RCC) has been previously defined within chromosome 3 by functional complementation experiments. The critical region of *NRC1* was further narrowed down to a 5-7cM region within 3p12 based on microsatellite analyses. The exact proximal and distal boundaries of the critical region have been ambiguous, however, because of the lack of informative markers, as well as the poor quality of chromosome 3p12 physical map before the NCBI Build 33 physical map was released. We have recently established a formal statistical framework for relative quantification using real-time quantitative PCR (QPCR). A SYBR Green I based QPCR method was also developed, which not only provides a sensitivity of detecting subtle DNA copy number changes as small as 25 percent, but also allows the use of any single tagged sites (STS) as a mapping marker. The improved technology (termed STS-QPCR) provides a theoretical mapping resolution as high as approximately 10-bp. Using the STS-QPCR method, we precisely define the *NRC1* critical interval to a 4.627-Mb region within chromosome 3p12. Furthermore, the distal boundary of *NRC1* was mapped to a 38-Kb interval between the exon 3 and exon 4 of the *DUTTI* gene, which was proposed as a candidate tumor suppressor gene partly because *NRC1* suppresses tumor growth in vivo. Further mutational screening and gene expression analyses indicate that *DUTTI* is not involved in the tumor suppressor activity of *NRC1*.

**TBX3 and TBX3+2a are Functionally Distinctive in Inhibition of Senescence and are Overexpressed in a Subset of Breast Cancer Cell Lines.** *T. Huang<sup>1</sup>, W. Fan<sup>1</sup>, X. Huang<sup>1</sup>, C. Chen<sup>2</sup>, L. Cai<sup>1</sup>, M. Gu<sup>3</sup>, L. Feutch<sup>1</sup>, J. Gray<sup>2</sup>.* 1) Div Human Genetics, Univ California, Irvine, Irvine, CA; 2) Laboratory Medicine and Radiation Oncology, Cancer Genetics and Breast Oncology, UCSF Comprehensive Cancer Center, University of San Francisco, San Francisco, CA; 3) Department of Pathology, University of California, Irvine, CA.

TBX3 is a transcription factor of the T-box gene family. Mutations of TBX3 cause Ulnar-Mammary syndrome (UMS) in humans, an autosomal dominant disorder characterized by the absence or underdevelopment of the mammary glands and other congenital anomalies. Recently, it was found that TBX3 was able to immortalize mouse embryo fibroblast cells. In addition, TBX2, a homologue of TBX3, is very active in preventing senescence in rodent cells and was found to be amplified in some human breast cancers. This study examined the roles of TBX3 and one of its isoforms, TBX3+2a, which differs in the DNA binding domain and has additional 20 amino acids, produced by alternative splicing, in the middle of the DNA binding domain. We first examined the tissue expression and alternative splicing patterns of these two isoforms. We found that TBX3 and TBX3+2a are widely expressed and that the ratio between them varies from tissue to tissue in human, suggesting that alternative splicing could be tissue-specific. In contrast, the ratio between *tbx3* and *tbx3+2a* is very consistent in mouse tissues. The replication-defective retrovirus carrying TBX3 (pFB-Neo-TBX3) is able to immortalize mouse embryo fibroblast cells, while TBX3+2a (pFB-Neo-TBX3+2a) shows an acceleration of senescence, a functional difference that may be explained by the fact that these two isoforms may have different downstream targets. TBX3, but not TBX3+2a, is able to bind to the previously identified T-box binding site in a nuclear extract gel shift assay. A subset of human breast cancer cell lines overexpresses TBX3 and TBX3+2a. Our results indicate that TBX3 and TBX3+2a are functionally distinctive in inhibition of senescence of mouse embryo fibroblast cells and may play an important role in breast cancer formation.

**Dye-primer chemistry reveals a cryptic mutation: A case report.** *J-A. Dolling<sup>1</sup>, M.D. Speevak<sup>1</sup>, N. Carson<sup>2</sup>, S. Taylor<sup>3</sup>, H. Feilotter<sup>3</sup>, R. Carter<sup>4</sup>.* 1) The Credit Valley Hosp, Mississauga, ON, Canada; 2) Children's Hospital of Eastern Ontario, Ottawa, ON, Canada; 3) Kingston General Hospital, Kingston, ON, Canada; 4) McMaster University Medical Centre, Hamilton, ON, Canada.

The BRCA2 mutation R2336P (G>C at 7235, NM\_000059) located at the end of exon 13 is predicted to adversely affect mRNA splicing and abrogate the generation of a full length, functional gene product. In the current study, we describe the testing parameters that led to the identification of this mutation in four family members and demonstrate a potential limitation of automated cycle sequencing using dye-terminated ddNTPs. Initial protein truncation test results showed an abnormal protein product derived from cDNA of exons 11-18. Automated cycle sequencing of genomic DNA using dye-terminator chemistry failed to identify the mutation. However, sequencing using dye-primer chemistry clearly detected the mutation in both the forward and reverse directions. The mutation was subsequently confirmed by allele-specific PCR. Reports in the literature have suggested that the context of the DNA sequence can affect electropherogram peak heights and ultimately detection of dye-terminated ddNTPs. With current dye-ddNTP terminator technology, suppression of peak heights has been minimized, and to our knowledge, there are no reports in which a dye-labeled ddNTP has gone completely undetected. For diagnostic purposes we therefore recommend further analysis of the genomic DNA sequence using dye-primer chemistry for cases in which the results of prescreening for mutations by another method are abnormal and reproducible.

**Mutational analysis of the MYH gene in familial adenomatous polyposis (FAP) patients negative for APC gene mutations.** *M.A. Blazo, M.R. Hegde, D.M. Odenbaugh, P.A. Ward, C.S. Richards.* Diagnostic Sequencing Laboratory, Molecular & Human Gen, Baylor College Medicine, Houston, TX.

Germline mutations in the APC gene are responsible for FAP and have been well characterized. The vast majority of mutations in the APC gene represent truncating mutations leading to frameshifts (small deletions, 46%; small insertions, 10%; nonsense mutations, 28%), although missense mutations (~3%) and gross alterations (~13%) have also been reported. Current technology is able to detect APC mutations in approximately 80% of the individuals who have received a clinical diagnosis of FAP. In our experience, approximately 65% of patients with a possible diagnosis of FAP are found to have an APC sequence variant that can be interpreted as a disease-causing mutation. Recently, two other genes, APC2 and MYH, have been investigated in patients with FAP phenotype. Germ-line mutations in the base-excision-repair gene MYH have been shown to be associated with recessive inheritance of multiple colorectal adenomas. Here we describe screening of 24 patients for mutations in the MYH gene who have a clinical diagnosis of FAP but have no identifiable mutation in the APC gene. The clinical presentation of these patients is typically presence of thousands of adenomatous polyps and in some cases extracolonic manifestations. We have evaluated the usefulness of denaturing high performance liquid chromatography (dHPLC) as a diagnostic tool for scanning the MYH gene for point mutations, small deletions, and insertions. The MYH gene was divided in 9 overlapping fragments to encompass the coding sequence and the splicing regions. All PCR reactions were amplified simultaneously using the same reaction conditions in a 96-well format and then analyzed by dHPLC, using empirically determined optimum temperatures for partial fragment denaturation. The assay was also performed and validated on dHPLC using 20 wild-type samples to scan for variants which were subsequently sequenced to identify SNPs.

**A high throughput, real-time PCR (TaqMan) method for detecting the heterozygous state for *GSTM1* and *GSTT1* deletion alleles.** *R. Welch*<sup>1</sup>, *S. Yadavalli*<sup>1</sup>, *V. Puri*<sup>1</sup>, *N. Rothman*<sup>2</sup>, *S.J. Chanock*<sup>3</sup>, *M. Yeager*<sup>1</sup>. 1) SAIC-Frederick, NCI-FCRDC, Frederick, MD at the Core Genotyping Facility, Department of Cancer Epidemiology and Genetics, NCI, NIH, Gaithersburg MD; 2) Occupational Epidemiology Branch, Department of Cancer Epidemiology and Genetics, NCI, NIH, Bethesda, MD; 3) Section on Genomic Variation, Pediatric Oncology Branch, NCI, NIH, Gaithersburg, MD.

Genes that are members of the glutathione-S- transferase family encode enzymes that are involved in the metabolism of a wide range of xenobiotics and carcinogens. Two of these genes, glutathione S-transferase M1 (*GSTM1*) and glutathione S-transferase theta 1 (*GSTT1*) have been implicated in the etiology of several cancers, including bladder and lung cancers. A characteristic of both of these genes is that they possess polymorphic gene deletions with significant heterozygosities. To date, the methods of genotyping these deletions has been by PCR or PCR-RFLP, which are not suitably sensitive to determine the heterozygous state, thus limiting the analysis to a strictly dominant model. We have developed separate, gene specific, high-throughput quantitative real-time PCR (TaqMan) assays that accurately and reliably produce genotypes for all three genotypic states (homozygous presence, heterozygous, and homozygous deletion). In the analyses of 16 CEPH family DNAs (N=232, performed in duplicate), and 102 CEPH unrelated individual DNAs of 4 self-described ethnicities (N=24 African/African Americans, N=31 Caucasians, N=23 Hispanics, N=24 Pacific Rim, all in duplicate) (<http://snp500cancer.nci.nih.gov>) there were no Mendelian inconsistencies in the family samples, no departure from Hardy Weinberg equilibrium in the unrelated individuals, and only a very small number of discordances in all duplicates (0.9 %). Further, we analyzed a SNP within *GSTT1* deleted region (UTR 101 bp 3 of STP), and all SNP/deletion combinations were as expected. Here, we present a novel method for genotyping two widely studied genes, including the determination of heterozygote state.

**DNA damage and repair in breast cancer.** *J. Blasiak*<sup>1</sup>, *M. Zadrozny*<sup>2</sup>, *K. Wozniak*<sup>1</sup>, *R. Krupa*<sup>1</sup>, *J. Drzewoski*<sup>3</sup>, *Z. Morawiec*<sup>4</sup>. 1) Dept. of Molecular Genetics, University of Lodz, Lodz, Poland; 2) Polish Mothers Memorial Hospital, Lodz, Poland; 3) Dept. of Clin. Pharmacol., Medical University of Lodz, Lodz, Poland; 4) N. Copernicus Hospital, Lodz, Poland.

Impaired DNA repair may fuel up malignant transformation due to accumulation of spontaneous mutations in target genes and increasing susceptibility to exogenous carcinogens. Moreover, the effectiveness of DNA repair may contribute to failure of chemotherapy and resistance of breast cancer cells to drugs and radiation. An individual ability to remove DNA damage may be, at least in part, determined by the genotype of DNA repair genes. It is generally accepted that the breast cancer susceptibility genes *BRCA1* and *BRCA2* are involved in DNA repair. To evaluate further the role of DNA repair in breast cancer we determined: 1) the kinetics of removal of DNA damage induced by UV light, gamma radiation, hydrogen peroxide and the anticancer drug idarubicin 2) the level of basal, oxidative and alkylative DNA damage before and after chemotherapy 3) polymorphisms of DNA repair genes: *ERCC1*, *RAD51*, *hOOG1*, *XRCC1*, *XRCC3*, *XPD* in the peripheral blood lymphocytes of breast cancer patients and healthy individuals. We observed slower kinetics of DNA repair after treatment by both kinds of radiation and idarubicin in lymphocytes of breast cancer patients compared to control individuals. The level of basal, oxidative and alkylative DNA damage was higher in breast cancer patients than in the control and the difference was more pronounced when patients after chemotherapy were enrolled. We found associations between the genotypes of *RAD51*, *hOOG1*, *XRCC1*, *XRCC3* repair genes and appearance of breast cancer. We did not find any link between genotypes of DNA repair genes and breast cancer progression. Our results indicate that polymorphisms in DNA repair genes may contribute to the appearance of breast cancer and the extent of DNA damage in breast cancer patients, especially those after chemotherapy, is significantly higher than in healthy individuals. The question whether decreased efficacy of DNA repair belongs to the reasons or is a consequence of breast cancer remains open.

**A new founder mutation (MSH2 deletion exon 8) in hereditary non-polyposis colorectal cancer families from Newfoundland with a distinct clinical phenotype.** *J. Green<sup>1</sup>, M. Woods<sup>1,2</sup>, D. Robb<sup>1</sup>, A. Pollett<sup>2</sup>, S. Gallinger<sup>2</sup>, B. Bapat<sup>2</sup>, J. McLaughlin<sup>2</sup>, P. Parfrey<sup>1</sup>, B. Younghusband<sup>1</sup>.* 1) Dept Medical Genetics, Memorial Univ Newfoundland, St John's, NF, Canada; 2) Mt. Sinai Hospital, Toronto, ON, Canada.

Hereditary non-polyposis colorectal cancer (HNPCC) is the most common form of hereditary colon cancer. Mutations in at least five known susceptibility genes involved in mismatch repair (MMR) have been identified in HNPCC families, the majority of these mutations being in MLH1, MSH2, and MSH6. The island of Newfoundland has a unique genetic structure because of historic and geographic factors. The majority of ancestors were from the southwest of England or the south of Ireland. Because of settlement in isolated coastal communities, large family size, and descendants living in neighboring areas, there are multiple genetic isolates with founder effect for many genetic disorders identified. Four HNPCC families with ancestors from a common geographic area, have a unique clinical phenotype with frequent transitional cancers of the renal pelvis, ureter, and bladder, as well as the more common HNPCC cancers including colorectal, gastric, endometrial, and ovarian cancers. A mutation was not identified by sequencing MLH1, MSH2, and MSH6, however a tumour from one affected individual was MSH2-deficient on immunohistochemistry. RT-PCR identified a deletion of MSH2 exon 8 which was confirmed as a genomic deletion by MLPA (Multiplex Ligation-Dependent Probe Amplification). This was confirmed in 11 affected members from the four families with the common phenotype from the same genetic isolate. A previous founder mutation (MSH2, 943+3 A to T) was identified in another cluster of 13 families from a distinct genetic isolate. In this previous founder cluster with greater than 150 affected individuals, colorectal, endometrial, and ovarian cancers were frequent but transitional cell cancers of the urogenital system were not identified.

**The influence of modifier genes on the disease phenotype in South African families with hereditary nonpolyposis colorectal cancer (HNPCC).** *R. Felix<sup>1</sup>, N. Fearnhead<sup>2</sup>, R. Ramesar<sup>1</sup>, W. Bodmer<sup>2</sup>.* 1) Division of Human Genetics, University of Cape Town Medical School, Cape Town, South Africa; 2) ICRF Cancer and Immunogenetics Laboratory, Weatherall Institute of Molecular Medicine, Oxford University, United Kingdom.

It is becoming increasingly apparent, in the field of human genetics that few diseases are caused exclusively by a mutation in a single gene. Cancer development and progression is a multiplex process involving numerous primary and modifier genes. A modifier is an inherited variation that leads to a qualitative or quantitative difference in the disease phenotype. As the knowledge of the influence and involvement of these primary and secondary cancer genes increases, it should be possible to accurately predict an individual's risk of developing the disorder as a function of both severity of the disorder and the age of onset. Individuals from HNPCC families with identical mutations in the hMLH1 and hMSH2 genes do not all exhibit the disease phenotype in the same manner. Differences exist in the age of onset of disease symptoms, the site of the cancer in the colon and the re-occurrence of cancer after treatment. It is postulated that polymorphisms and mutations within modifier genes could contribute to variability in the disease phenotype observed within these HNPCC families. Recently, several modifier genes have been shown to have a modifying role in colorectal cancers (CRCs). A cohort of 96 HNPCC individuals was investigated in the current study. These individuals harbour the same nonsense mutation (C1528T) in exon 13 of the hMLH1 gene and belong to 13 different families. Forty-three percent of these individuals had cancer of the proximal colon and eighty-six percent were diagnosed with CRC under 50 years of age. These subjects were screened for modifying DNA alterations in the CDH1, APC, ATM, CCND1, MTHFR, GSTT1, GSTM1 and the NAT2 genes. Statistical analyses were performed to identify any significant associations between these polymorphisms and the various phenotypes described in an attempt to explain the clinical variability observed in these subjects.

**Germline *Bcl-2* SNPs, haplotypes, and inherited predisposition to prostate cancer.** A. Coulibaly<sup>1</sup>, L. Long<sup>1</sup>, W. Chen<sup>1</sup>, R. Panguluri<sup>1</sup>, T. Lewis-Smith<sup>1</sup>, C. Bonilla<sup>1</sup>, W. Isaacs<sup>2</sup>, R.A. Kittles<sup>1</sup>. 1) National Human Genome Center, Howard University, Washington, DC; 2) Dept. of Urology, Johns Hopkins University, Baltimore, MD.

The expression of the anti-apoptotic protein Bcl-2 is strongly correlated with increased prostate tumor progression. The *Bcl-2* gene promoter consists of several transcription regulatory elements which may affect Bcl-2 expression. In order to determine if sequence variation of the *Bcl-2* promoter region affects prostate cancer risk we screened approximately 1.5 kb of the *Bcl-2* gene promoter and its two exons (total 717 bp) in 50 African American men using denaturing high performance liquid chromatography. Four single nucleotide polymorphisms (SNPs), -938C/A, -758 delT, -40C/T and +20A/G, were detected and confirmed by DNA sequencing. The -938C/A, -40C/T and +20A/G SNPs were genotyped using Pyrosequencing in three clinical populations consisting of African American (N=390), Jamaicans (N=205), and European American (N=210) prostate cancer cases and age and ethnicity matched controls. Haplotypes were generated using the EM algorithm. Association analyses were performed by contingency and regression analyses using single markers and haplotypes. Pairwise linkage disequilibrium (LD) was estimated for each population. We observed a reduced risk for prostate cancer among European-Americans for -938 AA genotypes (OR=0.25; 95%CI=0.09-0.64). Since the three SNPs were highly correlated (strong LD) in all populations (P<0.001), haplotype analysis was warranted. Haplotype analysis revealed the ACG haplotype to be significantly more frequent in European-American controls than in the cases implying that individuals with this haplotypes were at less risk for prostate cancer (OR=0.66, CI=0.44-0.98). African-American men with the CCA haplotype had an increased risk for prostate cancer (OR=1.39; CI=1.02-1.89). These data suggest that association analyses on closely linked SNPs are not independent and that combining the data into a haplotype analyses is a more powerful approach. Our haplotype analyses provide strong evidence that germ-line polymorphisms of the *Bcl-2* gene likely contribute to a prostate cancer risk.

**Multigene Combinations to Estimate Individual Risk for Breast Cancer.** *C. Aston*<sup>1,3</sup>, *D. Ralph*<sup>2</sup>, *E. Jupe*<sup>2</sup>, *C. Shimasaki*<sup>2</sup>, *J.J. Mulvihill*<sup>3</sup>. 1) Dept Arthritis & Immunology, Oklahoma Med Res Foundation, Oklahoma City, OK; 2) InterGenetics Inc, Oklahoma City, OK; 3) Oklahoma Univ Health Sci Ctr, Oklahoma City, OK.

In addition to BRCA1/2, variants of many other genes alter the risk for human breast cancer (BrCa), as seen in case-control studies. OMIM (20 June 2003) had 321 entries that mention BrCa. Against this background Pharoah et al (Nat Genet 2002;31:33) theorized that a panel of approx. 13 genes, acting without interaction, would be able to predict high risk individuals. We posit that there are interactions between genes that result in alterations in BrCa risk not predicted by the individual effects of the genes. To put form to theory, we developed SNP assays for polymorphisms in 9 genes that, individually, have been associated with increased relative risks of BrCa, such as prohibitin, cyclin D1, CYP17, and XPD. Polymorphisms were selected to have been replicated independently, have physiological consequences, and involve pathways of tumorigenesis. From a group of 1190 cases and 2584 controls, we report results for the Caucasian subsample. DNA from 770 women with incident BrCa diagnosed at ages 19 to 86 years was collected in Oklahoma, as well as from 1943 controls (21% with a 1st- or 2nd-degree relative with BrCa), ages 15 to 92 years. Allele frequencies at individual loci were verified to be in Hardy-Weinberg equilibrium in controls. For proof of principle and computational ease, all combinations of the 9 genes taken 3 at a time were assayed. Whereas the relative risks for individual genes ranged from 0.6 to 1.8; estimates for predisposing triads of markers were as high as 5.6 for the triad CYP17,COMT,SULT1A1 in pre-menopausal, and 5.1 for the triad VDR/ApaI,CYC D1,XPD 751 in post-menopausal women, and as low (protective) as 0.4 for a different allele triad for VDR/ApaI,CYC D1,XPD 751 in pre-menopausal women. Based on the individual gene effects and the non-interaction approach of Pharoah et al, the predicted relative risk for the triad CYP17,COMT,SULT1A1 in pre-menopausal women is 1.5 compared to the observed 5.6. These findings will enable stratification of BrCa risk across a wide spectrum, improving clinical risk assessment for all women.

**Inactivation patterns of *NF2* and *DAL-1* in sporadic meningioma.** *F.P. Nunes*<sup>1</sup>, *Y. Niida*<sup>2</sup>, *R. Beauchamp*<sup>2</sup>, *A.O. Stemmer-Rachamimov*<sup>3</sup>, *V. Ramesh*<sup>2</sup>, *J. Gusella*<sup>2</sup>, *M. MacCollin*<sup>1</sup>. 1) Neurology Department, MGH, Charlestown, MA; 2) Molecular Neurogenetics Unit, MGH, Charlestown, MA; 3) Neuropathology Department, MGH, Charlestown, MA.

Meningiomas are common neoplasms of the brain and spinal cord, accounting for 15-20% of all sporadic tumors of the human nervous system. The molecular genetic basis of the genesis and progression of sporadic meningioma remains unclear. We studied 63 sporadic meningiomas from adult patients. Intragenic and flanking markers at the *NF2* locus on chromosome 22 and the related cytoskeletal element *DAL-1* on chromosome 18 were used to detect loss of heterozygosity (LOH). We further sought to correlate the molecular genetic events with tumor pathology and grade. Forty-one of 62 informative tumors showed LOH at the *NF2* locus (67%) while only 12 of 60 informative tumors showed loss at the *DAL-1* locus (20%). Eleven of 12 tumors with *DAL-1* LOH also had *NF2* LOH. LOH of *NF2* and *DAL-1* was more common in malignant tumors than in atypical tumors, and was least common in benign tumors. This correlation was true when markers were analysed separately or together. *NF2* LOH was present in 100% of tumors classified as fibroblastic, 26% classified as transitional and 33% classified as meningothelial. In conclusion, we found the *DAL-1* locus to be less commonly involved in sporadic meningiomas than previously suggested. Ninety one percent of tumors with *DAL-1* loss also had *NF2* LOH, suggesting that *DAL-1* LOH may be a progression event rather than an early event in meningioma formation. Another tumor suppressor gene responsible for genesis of sporadic meningioma is yet to be identified.

**Apolipoprotein E gene polymorphisms in patients with breast cancer.** A. Sazci<sup>1</sup>, E. Ergul<sup>1</sup>, Z. Canturk<sup>2</sup>, Z. Utkan<sup>2</sup>.  
1) Dept Medical Biol & Genetics, Fac Med, Kocaeli Univ, Kocaeli, Turkey; 2) Dept Gen Surgery, Fac Med, Kocaeli Uni, Kocaeli, Turkey.

To investigate the role of apolipoprotein E (APOE) gene polymorphisms in breast cancer patients, we analysed the APOE genotypes of 107 patients and 193 controls. The results of a Chi-square analysis showed that APOE alleles were significantly distributed ( $\chi^2=11.526$ ;  $P=0.042$ ) between breast cancer cases and controls. The APOE 3 allele was statistically significant ( $OR=8.933$ ;  $95\% CI=1.163/68.592$ ;  $\chi^2=6.374$ ;  $P=0.012$ ). Nevertheless the APOE 4 allele had a protective effect towards breast cancer ( $OR=0.330$ ;  $95\% CI=0.148-0.736$ ;  $\chi^2=7.908$ ;  $P=0.005$ ). On the basis of genotypes, the APOE 33 genotype was 2.080-fold increased risk for breast cancer ( $OR=2.080$ ;  $95\% CI=1.185/3.651$ ;  $\chi^2=6.648$ ;  $P=0.010$ ). However the APOE24 genotype was protective against breast cancer ( $OR=0.938$ ;  $95\% CI=0.904/0.973$ ;  $\chi^2=6.930$ ;  $P=0.008$ ). The allelic frequencies of APOE 2, 3, 4 alleles were 10.36%, 79.27% and 10.36% in the controls and 6.07%, 89.72% and 4.21% in the breast cancer cases respectively. In conclusion, we observed an association among APOE alleles and genotypes and breast cancer.

**The role of *CHEK2* and *MSR1* genes in prostate cancer predisposition in Finland.** *E.H. Seppälä<sup>1</sup>, T. Ikonen<sup>1</sup>, V. Autio<sup>2</sup>, N. Mononen<sup>1</sup>, A. Rökman<sup>1</sup>, M.P. Matikainen<sup>3</sup>, T.L.J. Tammela<sup>3</sup>, J. Schleutker<sup>1</sup>.* 1) Laboratory of Cancer Genetics, Institute of Medical Technology, University of Tampere and Tampere University Hospital, Tampere, Finland; 2) Tampere School of Public Health, University of Tampere, Tampere, Finland; 3) Department of Urology, Tampere University Hospital and Medical School, University of Tampere, Tampere, Finland.

Recently, mutations in *CHEK2* (22q12.1) and *MSR1* (8p22-23) genes have been reported to associate with prostate cancer (PRCA) risk. In the present study from Finland, five sequence variants were identified in the SSCP analysis from the both genes in 120 index patients from Finnish families with hereditary prostate cancer (HPC). We found that the frequency of *CHEK2* 1100delC, a truncating variant that abrogates the kinase activity, was significantly elevated among 120 patients with HPC (4/120 [3.3%]; odds ratio [OR] 8.24; 95% confidence interval [CI] 1.49-45.54; P = 0.02) compared to 480 population controls. Suggestive evidence of segregation between the 1100delC mutation and prostate cancer was seen in all positive families. In addition, *CHEK2* I157T variant had significantly higher frequency among HPC patients (13/120 [10.8%]; OR = 2.12; 95% CI 1.06-4.27; P = 0.04) than the frequency 5.4% seen in the population controls. In contrast, when the carrier frequencies of the *MSR1* R293X, P275A, and -14743A>G variants were compared between the probands with HPC, unselected PRCA cases, and healthy male blood donors, no significant differences were observed. However, the mean age at diagnosis of the R293X mutation carriers among HPC probands was significantly lower compared to non-carriers (55.4 vs. 65.4 years; P = 0.04). The same trend was observed among unselected PRCA cases (65.7 vs. 68.7 years; P = 0.37). The results indicate that *CHEK2* variants are low-penetrance PRCA predisposition alleles that contribute significantly to familial clustering of PRCA at the population level. For the *MSR1* gene no major role in the causation of hereditary or unselected PRCAs is supported, but a possible modifying role in cancer predisposition is suggested.

**Oncogenic pathway of nasal NK/T cell lymphoma with novel germ line missense mutation in the *IGF2R* gene.**  
*H.S. Sun*<sup>1,2</sup>, *S.-M. Lin*<sup>1</sup>, *J.-S. Chen*<sup>2</sup>. 1) Inst Molecular Medicine, National Cheng Kung Univ, Tainan, Taiwan; 2) Inst Basic Medical Sciences, National Cheng Kung Univ, Tainan, Taiwan.

The human mannose 6-phosphate / insulin-like growth factor 2 receptor (M6P/IGF2R) gene encodes a multifunctional protein involved in lysosomal enzyme trafficking, fetal organogenesis, latent TGF- activation, and cytotoxic T cell-induced apoptosis. IGF2R gene contains 48 exons and spans 137kb genomic region on chromosome 6q25-27. Previous studies suggested that IGF2R acts as a tumor suppressor (TS) gene and sequence mutations have been identified in 60% of dysplastic liver lesions and HCCs, also in the early stage of breast cancer development. Furthermore, a poly-G region of the IGF2R gene was commonly mutated in colon, gastric and endometrial tumors with mismatch repair deficiencies and microsatellite instability. Result from our previous work has mapped potential TS gene containing region of nasal NK/T cell lymphoma to chromosome 6q25. IGF2R is a good candidate gene within this region thus it merits further investigation. This study aims to investigate if sequence variant of IGF2R gene plays a role in tumorigenesis for human nasal NK/T cell lymphoma. Ten exons of the IGF2R gene, which has been previously reported containing mutation hot spots, were selected for screening sequence variant. We have sequenced DNAs from 7 paired tumor and normal tissues of patients with the nasal NK/T cell lymphoma. One point mutation as well as several polymorphisms have been identified. The novel exon38 G5716A point mutation changes amino acid sequence from cysteine to tyrosine (Cys1856Tyr). Prediction of this protein sequence alteration has demonstrated a structural modification thus it can lead to functional change of the IGF2R protein. Extensive biochemical and molecular biological studies are currently undertaken to illustrate the potential role of this novel missense mutation on tumorigenesis of nasal NK/T cell lymphoma.

**Identifying novel homozygous deletions by microsatellite analysis and characterization of a putative tumor suppressor gene, TSG-9, on chromosome 9p in human lung cancer.** *Z. Shan, P. Tracy, J. Wiest.* Laboratory of Cellular Carcinogenesis and Tumor Promotion, CCR/NCI, Bethesda, USA.

Lung cancer is a worldwide medical problem and the leading cause of cancer-related mortality in the United States. Loss of heterozygosity studies show that genetic alterations of chromosome 9p occur in a variety of tumor types, suggesting the presence of tumor suppressor genes (TSGs) critical in the development of cancer. The p16/CDKN2 locus is located on 9p21 and is a major TSG inactivated in various cancers. We previously identified a region of homozygous deletion (HD) on chromosome 9p in lung cancer at D9S126, a locus distinct from the p16/CDKN2A gene. This finding led us to propose that chromosome 9p may harbor other TSGs important in lung tumorigenesis. To this end, 42 lung cancer cell lines were screened with 42 STS markers to search for new regions of homozygous on chromosome 9p. Four novel HD regions were identified in three lung cancer lines. The sizes of deleted regions vary from 980 Kb to 4.4 Mb. One gene identified in a HD, TSG-9, is a novel intronless gene. The deletion of TSG-9 was confirmed by PCR and Southern blot. Northern blot analysis of TSG 9 demonstrated two transcripts of approximately 2 kb (TSG-9L) and 1.5 kb (TSG-9S) that are ubiquitously expressed in various human tissues. Sequence analyses of TSG-9 identified alternative polyadenylation signals generating the two transcripts. However, both transcripts encode a peptide of 209 amino acids. Analysis of cDNAs from tumor cell lines demonstrated that TSG-9 is down regulated in lung cancer cell lines and hepatocellular carcinoma with/without homozygous deletion of the region, suggesting that TSG-9 may play the role in lung and liver tumorigenesis. To further address its function as a TSG, an expression vector containing TSG-9 will be transfected into the cell lines lacking expression of TSG-9 to study its effect on cell growth and tumorigenicity. The results will provide detailed information as to the function of TSG-9 as a tumor suppressor gene. Taken together, our results demonstrate several novel regions of HD and at least one gene within these regions may function as a TSG in lung and liver cancer.

**Mutation and segregation analysis of ATM alleles in 192 Australian breast cancer families.** *T. Wayne<sup>1</sup>, G. Chenevix-Trench<sup>5</sup>, M. Jenkins<sup>2</sup>, T. Bachrich<sup>3</sup>, D. Muhr<sup>3</sup>, R. Davis<sup>1</sup>, N. Albert<sup>4</sup>, P. Oefner<sup>1</sup>, Y. Thorstenson<sup>1</sup>.* 1) Stanford Genome Technology Center, Palo Alto, CA; 2) University of Melbourne, Australia; 3) University of Vienna, Austria; 4) University of Kiel, Germany; 5) Queensland Institute of Medical Research, Australia.

Mutations in the two major breast cancer susceptibility genes, BRCA1 and BRCA2, account for only 20 to 50% of families at high risk for the disease. The ATM gene is a third candidate for breast cancer susceptibility based on previous epidemiological studies. Although some reports failed to find a link between ATM mutations and breast cancer, studies with new, more sensitive technologies such as DHPLC cast doubt on those negative results. In our previous study of 270 Austrian families with Hereditary Breast and Ovarian Cancer (HBOC), 3.7% had deleterious ATM mutations. However, it was not possible to measure the penetrance of these alleles without further family data. Here, we report on the mutation analysis of 192 Australian families with an average of 5 samples per family. With about 50% of the gene analyzed to date, two mutations were observed in five families (2.5%). In addition, the most common missense variant previously observed in breast cancer families, L1420F, was observed in four families. Pedigree analyses will be performed to determine if these alleles segregate with breast cancer.

**Results of a genome-wide linkage analysis in prostate cancer families ascertained through the ACTANE Consortium.** *D. Easton.* CR UK Genetic Epidemiology, Univ Cambridge, Cambridge, United Kingdom.

THE INTERNATIONAL ACTANE CONSORTIUM Familial aggregation of prostate cancer suggests an inherited component. We performed a genome-wide linkage search in 64 families, 63 with at least 3 prostate cancer cases, from 5 countries. Most cases had clinically detected disease. 397 polymorphic markers were typed. Multipoint heterogeneity analysis was conducted under 3 genetic models and non-parametric analyses were undertaken. Weak evidence of linkage, under at least one model, was seen on chromosomes 2 (HLOD=1.15,  $\alpha=0.31$ ), 3 (HLOD=1.25,  $\alpha=0.36$ ), 4 (HLOD=1.28,  $\alpha=0.41$ ), 5 (HLOD=1.20,  $\alpha=0.29$ ), 6 (HLOD=1.41,  $\alpha=0.35$ ) and 11 (HLOD=1.21,  $\alpha=0.32$ ) and in two regions on chromosome 18 (HLOD=1.40,  $\alpha=0.30$  and HLOD=1.31,  $\alpha=0.32$ ). No HLOD scores were greater than 1.5 under any model, and no locus explains more than half of the genetic effect. There was no evidence in previously reported linkage regions on chromosomes 1, 8, 17, 20 or X. Genetic susceptibility to prostate cancer is likely to be controlled by many loci, with no single gene explaining a large fraction of the familial risk. Pooling of results from all available genome scans is likely to be required to obtain definitive linkage results. UK S Edwards, J Meitz, Q Hope, S Bullock, R Hamoudi, A Ardern-Jones, C Southgate, A Dowe, D Dearnaley, Cancer Research UK/BPG UK Collaborators, BAUS Section of Oncology, R Eeles+ Institute of Cancer Research. C Evans, D Teare, D Thomson, D Easton+ Cambridge Australia J Hopper+, G Giles+, D English, M Southey Cancer Epidemiology Centre, The Cancer Council Victoria, University of Melbourne, Centre for Genetic Epidemiology Canada W Foulkes+, N Hamel, S Narod, J Simard+. McGill University, Montreal and CHUL Research Center, Quebec City, and Center for Research in Women's Health, University of Toronto USAM Badzioch+, Chris Amos MD Anderson Cancer Centre, Texas. University of Washington Medical Centre, Seattle Norway K Heimdal, L Mahle+, P Moller+ Norwegian Radium Hospital, Oslo, Norway N Wessel, T Andersen+ Ullevaal University Hospital, Norway EU Biomed Tim Bishop+, Leeds UK+ PIs.

**Linkage and association studies localize prostate cancer aggressiveness gene on 7q32.** *J.S. Witte<sup>1</sup>, P. Curran<sup>2</sup>, M. Cicek<sup>2</sup>, B.K. Suarez<sup>3</sup>, J.K. Burmester<sup>4</sup>, W.J. Catalona<sup>5</sup>, G. Casey<sup>2</sup>.* 1) Dept of Epidemiology and Biostatistics, UC San Francisco, CA; 2) Dept of Cancer Biology, Cleveland Clinic Foundation, OH; 3) Depts of Psychiatry and Genetics, Wash Univ, St Louis, MO; 4) Center for Personalized Medicine, Marshfield Clinic Res Found, WI; 5) Dept of Urology, Northwestern University, Chicago, IL.

The severity of prostate cancer varies widely: some tumors progress to invasive life-threatening disease while others remain indolent for many years. Genetic factors may underlie this variability, and previous studies have detected linkage between 7q32 and Gleason score (a measure of tumor aggressiveness). We have further investigated this with linkage and association studies. In particular, we first undertook a new genome-wide linkage study of Gleason score (N=259 affected brothers). Second, we genotyped and analyzed an additional 101 finely spaced markers in the 259 men, and in 594 previously studied brothers, allowing for a pooled genome-wide analysis of 853 affected brothers. We detected linkage to chromosome 7q32 in the new study ( $p=0.0009$ ), and in our pooled analysis ( $p=0.0002$ ), confirming previous results. A biologically plausible candidate gene for tumor aggressiveness at this locus is podocalyxin-like protein (*PODXL*), a transmembrane sialomucin. Others have implicated *PODXL* as a down stream target of Ets-1, a positive regulator of angiogenesis, and suggest that *PODXL* is regulated by the tumor suppressor gene *WT1*. Therefore, we next searched for mutations in *PODXL* among 17 men exhibiting linkage, and detected an in-frame deletion. We then evaluated the association between the deletion and prostate cancer aggressiveness with a sibling-based case-control study (N=920). In comparison with carrying no deletions, men with one or two deletions had a 2.2- or 4.7-fold increased risk of aggressive prostate cancer, respectively ( $p=0.004$ ). This suggests that *PODXL* may be the aggressiveness gene linked to 7q32. Using information about such genes to distinguish tumor aggressiveness will provide valuable information about the most appropriate course of treatment among men diagnosed with prostate cancer.

**Enhancing minority recruitment into a national genetics registry: Data from the Cancer Genetics Network.** *D. Bowen, T. Vu.* Cancer Genetics Network, Minority Recruitment Committee, Seattle, WA.

The Cancer Genetics Network (CGN) is a national collaboration at 8 sites in the US, all focused on the conduct of studies on genetic influences on cancer incidence and mortality. The majority of the CGN participants (n=18,000) are Caucasian (90%), with small proportions of Hispanic (4%), African American (3%), Asian (1%) and other ethnicities (2%). To enhance the recruitment of minority individuals and families into the CGN, the investigators have designed four protocols to test new strategies of recruitment. Cluster 1 focused on testing a culturally tailored brochure in African American families in a randomized trial. Cluster 2 is testing the use of genetic risk assessment as an incentive to participate, also in African American participants. Cluster 3 is testing a Spanish language, culturally targeted magazine, Buena Vida, as a novel communication strategy for Hispanic participants in a randomized trial. Cluster 4 tests two incentives, a pan-Asian welcome letter and the provision of a telephone card, to enhance recruitment from the federally funded cancer registry into the CGN.

To date, these efforts have resulted in over one thousand new minority contacts for the CGN. Participants have come from medical, community, and registry settings. All four studies are in their final stages and will be completed in August, 2003. These studies will inform the scientific community about the best ways to recruit minority individuals into cancer genetics studies.

**Microfluidic analysis of multiplex reverse transcription-PCR Assays for semi-quantitative gene expression screening.** *R. Salowsky<sup>1</sup>, R. Wittig<sup>2</sup>, J.S. Maa<sup>3</sup>, M.J. Su<sup>3</sup>, S. Blaich<sup>2</sup>, J. Mollenhauer<sup>2</sup>, A. Poustka<sup>2</sup>, O. Mueller<sup>1</sup>.* 1) Agilent Technologies, Waldbronn, Germany; 2) Department of Molecular Genome Analysis, German Cancer Research Center, Heidelberg, Germany; 3) Maxim Biotech, San Francisco, CA.

During the last decade, micro-array analysis led to the identification of a large number of genes that are differentially expressed in disease processes. After target validation, identified marker genes can be used for screening of different samples (e.g. biopsies, cell lines, blood) in clinical research. Multiplex PCR (mPCR), including the amplification of different DNA fragments in one single reaction, has become a versatile experimental tool for the gene expression screening via reverse transcription-PCR (RT-PCR). For the analysis of multiple bands of an mPCR experiment, the data acquisition from slab gels is often inconvenient in terms of sizing resolution and data accuracy. To advance the analytical parameters of mPCR studies, a microfluidic based analytical platform using Lab-on-a-chip technology was tested to replace slab gels as the tool for the electrophoretic separation of the mPCR products. For the semi-quantitative monitoring of expression variances in different cancer cell lines, an mRT-PCR assay including 12 out of 266 target genes for cancer drug resistance was developed. These target genes were formerly identified by cDNA-array based expression analysis. Lab-on-a-chip technology was applied for the quality control and quantitation of RNA samples as well as for the electrophoretic separation, sizing and quantitation of the mRT-PCR products. All 12 amplicons ranging from 116 582 bp could be separated and quantified. The results of this study clearly demonstrate the improved sizing resolution and data accuracy of this technology compared to slab gels, thereby expanding the application areas for mPCR assays.

**Methylation of p15 tumor-suppressor gene in familial and sporadic malignant melanoma.** *G.A. Molfetta*<sup>1,2</sup>, *P.S.P. Lima*<sup>1,2,3</sup>, *M.V.S. Passos*<sup>1</sup>, *W.A. Silva-Jr*<sup>1,2</sup>. 1) Centro de Terapia Celular-Hemocentro de Ribeirao Preto-Brasil; 2) Depto. Genetica-FMRP-USP-Brasil; 3) Depto. Ciencias Naturais-UESB-BA-Brasil.

The malignant melanoma has its origin in abnormal melanocytes and, it is the worst type of cancer due to its high probability of metastasis. The malignant melanoma is mostly spread among white individuals with an important increase of its incidence worldwide. However, there are few data on the molecular basis of its origin. Hypermethylation of the CpG islands in the promoter region is the only mechanism for the loss of function of many tumours. Additionally, there are many tumor-suppressor genes that are silenced by promoter hypermethylation in cancer. With the objective of evaluating the p15 methylation in familial and sporadic malignant melanoma we have studied the p15 methylation status in 10 patients with familial melanoma and in other 10 patients with sporadic melanoma. To assess the p15 methylation status we used methylation-specific PCR technique. We have observed that 90% of the patients with familial melanoma showed unmethylated p15 pattern and, 80% of the patients with sporadic melanoma showed both methylated and unmethylated p15 patterns. It has been reported that p15 mutations play a role in the origin of sporadic melanoma; our results show that p15 methylation is also related to sporadic melanoma. In addition, we have observed that p15 methylation may occur in late stages of melanoma tumorigenesis. When comparing the p15 methylation status in familial and sporadic malignant melanoma, we concluded that the type of abnormality causing sporadic melanoma seems to involve more complex mechanisms. It seems that the abnormalities causing familial cases of melanoma are mutations in specific genes, while in the sporadic cases there is an accumulation of abnormalities in one or more genes, which act in conjunction for the regulation of the gene expression. Hypermethylation in sporadic melanoma is a consequence of other initial abnormalities in genes which control the normal pattern of methylation. P15 methylation is observed with a higher frequency in patients with sporadic melanoma in later stages, which reinforces our hypothesis. Financial support: FAEPA, FUNDHERP.

**EXPRESSION OF BREAST CANCER RESISTANCE PROTEIN DEPENDS ON THE METHYLATION STATUS OF ITS PROMOTER REGION IN LUNG CANCER CELLS.** *K. Tsukamoto<sup>1</sup>, M. Kamikatahira<sup>1</sup>, S.*

*Sado<sup>1</sup>, K. Yanagihara<sup>1</sup>, T. Kishino<sup>2</sup>, M. Oka<sup>3</sup>, S. Kohno<sup>3</sup>, I. Murata<sup>3</sup>.* 1) Div Pharmacotherapeutics, Dept Clin Pharm, Course Pharmaceutical Sci, Grad Sch Biomed Sci, Nagasaki Univ, Nagasaki, Jpn; 2) Nagasaki Univ Gene Res Center, Nagasaki, Jpn; 3) Div Mol & Clin Microbiol, Dept Mol Microbiol & Immunol, Grad Sch Biomed Sci, Nagasaki Univ, Nagasaki, Jpn.

Breast cancer resistant protein (BCRP) functions as a drug efflux transporter that causes resistance to certain anticancer drugs when expressed in cancer cells. Methylation of the CpG sites in the promoter region of the certain genes has been shown to cause their transcriptional silencing. SN-38 substrate used in this study is an active metabolite of irinotecan. Since the mechanism underlying expression of the BCRP gene during acquisition of drug resistance remains unclear, the aim of this study was to determine the methylation status of the BCRP promoter region in both small cell lung cancer cell line, PC-6 and its SN-38-resistant cell line, PC-6/SN2-5H. We screened the methylation status of the promoter region (spanning from nt -1381 to nt +261 with respect to the transcription initiation site) of the BCRP gene (GenBank accession No.: AF151530), compared to DNA sequences with or without bisulfite modification treatment for each cell line. We found that all of the CpG sites in this whole region examined were completely methylated in SN-38-sensitive cells, PC-6. On the other hand, in SN-38-resistant cells, PC-6/SN2-5H, some of the CpG sites in the limited region (nt -721 to nt -109) were unmethylated at both alleles. Our results indicate that the hypomethylation status of the BCRP promoter region might be necessary for BCRP expression, leading to drug resistance in this lung cancer cells. Furthermore, we have designed methylation-specific PCR (MSP) primer set in the hypomethylated region in order to determine the methylation status of the BCRP promoter region easily. At the next step, we will screen methylation status of the biopsy specimens of lung cancer before chemotherapy with irinotecan by MSP, implicating the possibility to predict irinotecan responsibility before chemotherapy.

***FANCF* Promoter is Methylated in Human Sporadic Breast Cancers.** M.J. Wei<sup>1</sup>, J. Dignam<sup>2</sup>, L. Sveen<sup>1</sup>, J. Fackenthal<sup>1</sup>, T. Taniguchi<sup>3</sup>, F. Hagos<sup>1</sup>, A.D. D'Andrea<sup>3</sup>, O.I. Olopade<sup>1</sup>. 1) Department of Medicine, The University of Chicago, Chicago, IL 60637; 2) Department of Health Studies, University of Chicago, Chicago, IL 60637; 3) Department of Pediatric Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

*FANCF*, a gene of Fanconi anemia (FA), encodes a protein required for DNA damage-inducible monoubiquitination of FANCD2 and for targeting of FANCD2 to DNA repair complexes that include the BRCA1 and BRCA2 proteins. A previous study suggested that methylation of CpG islands in the *FANCF* promoter regions in ovarian tumors results in *FANCF* gene silencing and cisplatin resistance. Previous work from our lab has shown that several cancer-related genes may be simultaneously silenced by methylation in breast tumors, including *BRCA1* and *ER*. We therefore sought to analyze the *FANCF* methylation status and to determine its relationship with *ER* and *BRCA1* promoters methylation, in primary breast cancers. Methylation patterns in the *FANCF*, *BRCA1* and *ER* promoter were assessed in breast cancer cell lines and primary breast tissues by methylation-specific PCR (MSP). *FANCF* RNA expression was determined in cell lines by reverse transcription PCR. To date, we have analyzed 5 breast cancer cell lines and 75 invasive breast cancers. The results show that CpG islands in the 5' region of the *FANCF* are methylated in 13 of 75 (17.3 %) of the primary breast cancers while *BRCA1* was methylated in 20 and *ER* in 33 of the same samples. Interestingly, *FANCF* methylation status was strongly correlated with *BRCA1* promoter methylation (50.0 % of *BRCA1* methylated tumors were *FANCF* methylated vs. 5.5 % *FANCF* methylation in *BRCA1* unmethylated tumors,  $P < 0.001$ ). There was no association between *FANCF* methylation status and *ER* methylation ( $P = 0.54$ ). Of the cultured human breast cancer cell lines, MDA-MB-231 cells exhibited methylated *FANCF*; *FANCF* mRNA was expressed, at a relatively low level. These data suggest that epigenetic mechanisms resulting in disruption of the Fanconi Anemia-BRCA pathway contribute to tumor progression in a significant proportion of primary breast tumors, and could be targeted for novel treatment and chemoprevention approaches.

**The Human Mitochip: A High-throughput Sequencing Microarray for Mitochondrial Mutation Detection. A.**

*MAITRA*<sup>1</sup>, *Y. COHEN*<sup>1</sup>, *S. GILLESPIE*<sup>1</sup>, *E. MAMBO*<sup>1</sup>, *N. SHAH*<sup>2</sup>, *D. SIDRANSKY*<sup>1</sup>, *A. CHAKRAVARTI*<sup>1</sup>. 1) PATHOLOGY, ONCOLOGY, AND GENETIC MEDICINE, JOHNS HOPKINS UNIVERSITY, BALTIMORE, MD; 2) AFFYMETRIX, INC., SANTA CLARA, CA.

Somatic mitochondrial mutations are common in human cancers, and can be used as a tool for early detection of cancer. We have developed a mitochondrial Custom Reseq microarray as an array-based sequencing platform for rapid and high-throughput analysis of mitochondrial DNA. The MitoChip contains tiled oligonucleotide sequencing probes synthesized using standard photolithography and solid-phase synthesis, and is able to sequence >29kb of double stranded DNA in a single assay. Both strands of the entire human mitochondrial coding sequence (15,451bp) are arrayed on the MitoChip; both strands of an additional 12,935bp (84% of coding DNA) are arrayed in duplicate, providing internal validation of sequence data. We used 300ng of genomic DNA to amplify the mitochondrial coding sequence in three overlapping long PCR fragments. We then sequenced >1.6 million base pairs of mitochondrial DNA, and successfully assigned base calls at 96.54% of nucleotide positions. Replicate experiments demonstrated >99.99% reproducibility. In serial dilution experiments using mixed normal and tumor DNA, the MitoChip was able to detect an aberrant clonal population in 50-fold diluted samples. Consequently, the MitoChip is a high-throughput sequencing tool for the reliable identification of mitochondrial DNA mutations from primary tumors in clinical samples.

**Mitochondrial DNA mutations and aging in human colonic crypts and stem cells.** *R.W. Taylor<sup>1</sup>, M.J. Barron<sup>1</sup>, G.M. Borthwick<sup>1</sup>, A. Gospel<sup>1</sup>, P.F. Chinnery<sup>1</sup>, D.C. Samuels<sup>2</sup>, G.A. Taylor<sup>1</sup>, S.M. Plusa<sup>3</sup>, S.J. Needham<sup>4</sup>, L.C. Greaves<sup>1</sup>, T.B.L. Kirkwood<sup>5</sup>, D.M. Turnbull<sup>1</sup>.* 1) Department of Neurology, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom; 2) Department of Mathematics, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom; 3) Department of Surgery, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom; 4) Department of Pathology, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom; 5) Department of Gerontology, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom.

Oxidative stress plays a major role in the build-up of molecular damage underlying the aging process. The main source of oxidative stress within cells is mitochondrial respiration, which generates reactive oxygen species (ROS) as by-products. Because mitochondrial DNA (mtDNA) is close to the site of ROS production and has limited repair capacity, it is especially vulnerable to stress-induced mutation. Several studies have reported age-related accumulation of mtDNA mutations in post-mitotic tissues such as muscle and brain. These mutations impair cellular energy generation, thereby contributing to degenerative processes in these tissues. To date, age-related pathogenic mtDNA mutations have not been described in actively dividing tissues.

Here we present data from human colonic epithelium showing a marked increase with aging of respiratory deficient colonic crypts. Within these crypts are pathogenic mtDNA mutations, which because of the unique crypt structure must originate in the stem cells. These observations highlight the potential impact of mitochondrial DNA mutations on aging of human stem cells. Our observations explain a recently reported association between mtDNA mutations and colon cancer, a late-onset condition for which age is the major risk factor, suggesting a connection between mitochondrial genome instability, aging, stem cells, and cancer.

**High throughput mitochondrial genome screen in non-melanoma skin cancer (NMSC) using temperature capillary gradient gel electrophoresis (TGCE).** *J.E. Sligh, W. Giraldo-Rosa.* Division of Dermatology, Vanderbilt University Medical Center, and VA TN Valley Healthcare System, Nashville, TN.

Mitochondrial DNA (mtDNA) mutations have been observed in a variety of human malignancies. Surprisingly, these mutations were often homoplasmic within the tumors. Theories to explain the role of these mtDNA changes in tumorigenesis have included a possible selective growth advantage or that they arise by stochastic segregation. We explored the utility of TGCE as a screening tool to identify mutations in human NMSC. TGCE is a high throughput method for detection of DNA sequence variation using capillary gel electrophoresis in a parallel array run through a controlled temporal temperature gradient and paired with laser-induced fluorescence detection. We obtained paired tissue specimens of NMSC tumors and adjacent tumor-free margin skin from excisions. DNA was isolated from the specimens for screening of the entire mtDNA by multiplexed TGCE. We amplified the entire mtDNA in 17 overlapping amplicons averaging 1.1 kb in size (range 418 bp to 2 kb) using a high fidelity DNA polymerase. Larger amplicons were digested with restriction endonucleases to generate up to five smaller fragments (as large as 588 bp). The PCR-amplified segments of the mtDNA from the tumors were annealed with the PCR products amplified from the margin skin, allowing for the formation of heteroduplexes in regions of DNA mismatch. Regions of the mtDNA that formed heteroduplexes were detected by the Reveal system (SpectruMedix) and compared to our sequencing data of the mtDNA. We identified a variety of polymorphisms as well as homoplasmic point mutations in the mtDNA including protein synthesis mutations affecting the mtDNA-encoded rRNA subunits and mutations affecting specific subunits of complex I and complex IV. The TGCE system was highly sensitive in detecting mutations present in amplicons less than 800 bp in both singlet reactions and in multiplexed assays. A mutation was also detected by TGCE in a 2 kb amplicon, although not in all amplicons of this size. We conclude that multiplexed TCGE is a sensitive and rapid screening tool for identifying mtDNA variation.

**NCI Mouse Models of Human Cancers Consortium - Resources for Discovery.** *C.L. Marks.* Division of Cancer Biology, National Cancer Institute, Bethesda, MD.

Mouse models are invaluable for exploring many aspects of the natural history and clinical course of human cancers. The development of new genetically engineered strains promises to provide increasingly accurate models for cancer researchers engaged in basic, translational, clinical, and epidemiological investigations. To accelerate the pace at which well-designed, appropriate mouse models are available for application to human cancer research, the NCI established the **Mouse Models of Human Cancers Consortium (MMHCC)**. The Consortium works cooperatively with the NCI to evolve an integrative program of human/mouse cancer research, convene community-based meetings to promote state-of-the-art mouse cancer science, and identify resources and infrastructure for cancer model development and application to basic and translational cancer research.

The **NCI Center for Bioinformatics** (<http://ncicb.nci.nih.gov>) provides the necessary infrastructure to integrate descriptive cancer models information with that from comparable human diseases. Enabling this information system to function are the **Cancer Models Database** and the **Cancer Images Database**; their data reflect broad experience with how well cancer models inform human therapy, prevention, early detection, imaging, and population science. The interface to the NCI's preclinical models programs is the **MMHCC Website** (<http://emice.nci.nih.gov>), an expanding resource of cancer models information and useful links.

The NCI deploys models to the research community from the **Mouse Models Repository**. The Repository maintains and distributes fully developed cancer models, as well as strains that may be used to derive models. The Repository, in Frederick, Maryland, serves the worldwide scientific community through its website at <http://mouse.ncifcrf.gov>. The site has descriptions of available strains and information about obtaining or submitting mouse models. The Repository monitors the genetic quality of both the mutated allele(s) and backgrounds for all strains, and cryopreserves sperm, and occasionally embryos or ovaries, from each strain. Breeding pairs are available from the Repository at no charge except for the cost of shipping.

**PUMA (P53 Upregulated Modulator of Apoptosis) induces free radicals generation in cancer cell death.** *H. Lu<sup>1</sup>, Z. Liu<sup>1</sup>, H. Shi<sup>2</sup>, J. Yu<sup>3</sup>, K. Liu<sup>2</sup>, C.A. HU<sup>1</sup>.* 1) Dept. Biochem. and Molecular Biol.; 2) Toxicology Program, Univ. New Mexico Health Sciences Center, Albuquerque, NM; 3) Dept. Pathology, Univ. Pittsburgh Cancer Institute, Pittsburgh, PA.

Many cancer cells exhibit aberrations in the genetic and biochemical pathways of apoptosis (programmed cell death). Apoptosis, which is defective in most cancers, is regulated by reactive oxygen species (ROS). Disturbances of ROS homeostasis are involved in the pathophysiology of cancer and other diseases such as stroke and atherosclerosis. Many chemotherapeutic drugs with diverse structures and acting on distinct primary targets induce cancer cell death through P53-mediated apoptosis. The tumor suppressor gene, p53, is mutated in >50% of all cancers and induces apoptosis, mainly through the generation of ROS. The Bcl-2 protein, an oncogene product possessing anti-apoptotic activity, may itself have significant antioxidant activity. In order to better understand the mechanisms underlying ROS generation and homeostasis in cancer cell death, we investigated functions of PUMA (P53 Upregulated Mediator of Apoptosis) in the induction of ROS generation in a Tet-Off inducible cancer cellular model, DLD-1.PUMA. PUMA, a novel mitochondrial Bcl2-homology domain 3 (BH3)-only protein and a potent p53 downstream pro-apoptotic effector, induces rapid apoptosis through a mitochondria-dependent pathway. Utilizing ROS scavengers, electron paramagnetic resonance (EPR) techniques and ROS-sensitive fluorescence dye analysis, we found that: (i) PUMA-induced apoptosis in DLD-1, a colorectal cancer cell line, is specific and is PUMA-dose-dependent; (ii) PUMA-induced apoptosis is directly related to ROS generation because ROS can be detected in apoptotic DLD-1.PUMA cells by EPR and fluorescence dye analysis; (iii) Diphenyleneiodonium chloride (DPI), a ROS scavenger, slows down apoptosis in DLD-1.PUMA cells; and (iv) Quantitative RT-PCR study shows that overexpression of PUMA in DLD-1.PUMA cells induces oxidative stress-responsive genes (e.g., Wild type p53-induced Gene 1, or Wig-1/PAG608, was induced >4X). In summary, we showed that PUMA induces apoptosis, in part, through the generation of ROS and oxidative stress.

**Germ-line mutations in multiple primary melanomas.** *C. Badenas*<sup>1,3</sup>, *J. Malvey*<sup>2</sup>, *M. Mila*<sup>1,3</sup>, *C. Herrero*<sup>2</sup>, *S. Puig*<sup>2,3</sup>, *The Melanoma Genetic Consortium*. 1) Genetics, Hospital Clinic, Barcelona, Barcelona, Spain; 2) Dermatology Service. Hospital Clinic. Barcelona. Spain; 3) IDIBAPS (Institut d'Investigacions Biomediques Agusti Pi i Sunyer). Hospital Clinic. Barcelona. Spain.

Cutaneous malignant melanoma (CMM) is a potential fatal type of skin cancer from melanocytes. Ten to 26% multiple primary melanoma patients (MPM) have been reported to carry mutations in the CDKN2A irrespective of family history. Approximately 5% of melanoma patients will develop more than one primary melanoma. We report the screening of 60 MPM patients (46 with 2 melanomas, 10 with 3 melanomas, 3 with 4 melanomas and 1 with 6 melanomas) for germ-line mutations in CDKN2A, p14ARF and CDK4 exon2. We have detected 7 different mutations in 13 cases (21.7%). Five mutations affect CDKN2A exon 2, 1 affects CDKN2A exon 1 and one affects p14ARF exon 1. No mutation has been detected in CDK4 exon 2. Two mutations are recurrent: G101W and R87W that are found in 6 and 2 patients, respectively. The only remarkable item is age at onset. Dysplastic nevi or family history does not show statistical differences between mutation and non-mutation groups. The mean age at diagnosis of the first melanoma in the mutation group is 31.4 years (range 19-66), while in the non-mutation group is 43.8 years (range 20-83). The results presented in this study show that more than 20% of MPM individuals carry CDKN2A mutations in the Spanish population. Acknowledgements: NIH (NI5.38226/H) and Red Nacional de Genetica Clinica Molecular (V2003redC07).

***In vivo* loss of heterozygosity in splenic T cells accumulates with age.** L. Deng<sup>1</sup>, L. Liang<sup>1</sup>, M. Kim<sup>1</sup>, C. Shao<sup>1</sup>, P. Stambrook<sup>2</sup>, J. Tischfield<sup>1</sup>. 1) Dept of Genetics, Rutgers Univ, Piscataway, NJ; 2) Dept of Cell Biology, Neurobiology, and Anatomy, Univ of Cincinnati, Cincinnati, OH.

The age-related increase in cancer is hypothesized to be the result of accumulation of mutations in somatic cells. Such mutations may lead to loss of function of heterozygous tumor suppressor genes. There are several mechanisms that produce this loss of heterozygosity (LOH), resulting in loss of cellular growth control and, ultimately, carcinogenesis. To test whether or not LOH accumulates with age, we have measured the mutant frequency at the *Aprt* (adenine phosphoribosyltransferase) locus and examined mechanisms of LOH in normal T cells of "old" (12-months-old, n = 19) and "young" (4-months-old, n = 47) *Aprt* heterozygous mice. T cell variants that arose *in vivo* as a consequence of genetic or epigenetic alterations abolishing APRT function were selected and expanded *in vitro* by virtue of their resistance to 2,6-diaminopurine. The *Aprt* mutant frequency increased significantly with age, from a median frequency of  $17 \times 10^{-6}$  in young mice to  $41 \times 10^{-6}$  in old mice. This 2.4-fold increase was caused by accumulation of mutants showing physical loss of the *Aprt* wild-type allele (class I mutants) as well as mutants that did not exhibit loss of the *Aprt* wild-type allele (class II mutants). Microsatellite marker and cytogenetic analyses of class I mutants indicated that mutational events, such as mitotic recombination, chromosome loss, and gene conversion/interstitial deletion, were increased in older mice. DNA sequencing analysis of class II variants showed that intragenic point mutations, predominantly base substitutions, increased during aging. Interestingly, epigenetic allelic inactivation also significantly increased, by about 4-fold, in older mice. Taken together, the data indicate that several different types of mutations resulting in LOH accumulate with age. This study improves our understanding of how accumulation of somatic mutations can contribute to various age-related diseases, including cancers.

**Mitotic Instability of Rare *HRAS1* Minisatellite Alleles in *S. cerevisiae*.** S. Ding<sup>1</sup>, G.P. Larson<sup>1</sup>, A.M. Bailis<sup>2</sup>, T.G. Krontiris<sup>1</sup>. 1) Divisions of Molecular Medicine; 2) Divisions of Molecular Biology; City of Hope Beckman Research Institute, Duarte, CA.

The human *HRAS1* minisatellite is located at 11p15.5 and is composed of 30-100 units of a polymorphic 28-bp repeat. Several dozen rare alleles, which arise from four common alleles as a consequence of both replication errors and gene conversion events, demonstrate a significant association with breast, colon, lung, and prostate cancer. To explore the pathways controlling the stability of the *HRAS1* minisatellite, we designed a plasmid-based assay to investigate and compare the alleles' spontaneous mitotic mutational events in *S. cerevisiae*. The primary transformation efficiency of 80 rare alleles was lower than that of 50 common alleles in a wild-type strain; but there was only a marginally significant difference ( $2.831.67 \times 10^3$  vs.  $3.512.24 \times 10^3$ ,  $p=0.049$ ). However, the length alteration of rare alleles was significantly higher than that of common alleles when genotyping individual colonies (28.1624.28% vs. 20.2716.65%,  $p=0.007$ ). Also, this ratio was increased dramatically after incubation of full-length allele clones in nonselective YPD media for 24 hours (62.7626.34% vs. 40.8120.05%,  $p=0.0001$ ). We also sequenced 30-50 randomly selected clones from each of wild type, *rad51* mutant, *dnl4* mutant, *rad1* mutant, and *rad51/dnl4* double mutant strains after YPD incubation. The DNA double-strand breaks repair consequences, such as crossovers and complex conversions, were observed after co-transformation of two rare alleles; but no such events were identified after co-transformation of one rare allele with one common allele. Compared with wild type, the mutation frequencies were slightly lower in *dnl4* (-4%,  $p=0.681$ ) and *rad1* (-18%,  $p=0.079$ ), but significantly dropped in *rad51* (-35%,  $p=0.0003$ ) and *rad51/dnl4* (-36%,  $p=0.004$ ) mutant strains. Therefore, Rad51 mediated strand exchange for homologous recombination repair initiated by spontaneous DNA double-strand breaks, which play a major role in the instability of rare *HRAS1* minisatellite alleles.

**Assessment of hMSH2/hMLH1 genomic rearrangements in individuals at risk for Hereditary Nonpolyposis Colorectal Cancer.** *P.J. Ainsworth, D. Koscinski, B.P. Fraser, J.A. Stuart.* Molecular Diagnostic Lab, London Hth Sci/Univ Western On, London, ON, Canada.

Hereditary Nonpolyposis Colorectal Cancer (HNPCC) results from inactivating germline mutations in a set of DNA mis-match repair genes, of which the most clinically relevant are hMSH2 and hMLH1. The calculation of an individual's likelihood of carrying such a deleterious mutation is typically carried out by assessment of the relevant pedigree, with attention to the number of related cancer-affected individuals, the type of HNPCC-associated cancers, and the age at diagnosis. More recently computer assisted risk assessment tools have become available to facilitate this type of risk analysis. One such tool, the CancerGene program obtained from Dr D. Euhus, University of Texas, SouthWestern, was used to assess the risk of colon cancer in a panel of 39 individuals referred to the LRCC Cancer Genetics Clinic because of their family history of HNPCC-related cancers. Of this panel, 22 individuals were calculated to have a >10% risk of colon cancer by the CancerGene method. DNA isolated from each member of the panel of 39 was screened for genomic re-arrangements in the hMSH2 and hMLH1 genes by the Multiplex Ligation-dependent Probe Amplification (MLPA) method that has recently become available as a kit from MRC-Holland. Individuals from 7 distinct families were determined to carry a genomic rearrangement in either hMSH2 (hMSH2del ex1\_8, (2)hMSH2del ex8, hMSH2del ex12\_16) or in hMLH1 (hMLH1del ex2\_6, (2)hMLH1del ex12). Six of the individuals with genomic rearrangements were calculated by CancerGene to have a colon cancer risk of >50%, the 7th individual being calculated to have a risk of >25%. Though the numbers in this pilot study are small, the trend is unmistakable. The proportion of hMSH2/hMLH1 inactivating genomic re-arrangements in individuals with a colon cancer risk of >10% (as calculated by CancerGene) is >30 %, a number consistent with the current literature. Furthermore genomic rearrangements invariably resulted in a more severe familial pattern of HNPCC. We suggest that the MLPA assay be utilised as an economical primary screen of probands calculated to be at risk for HNPCC.

**Fluorescent DHPLC assay for deletion screening: a preliminary study.** C. Dehainault, A. Laug, V. Caux-Moncoutier, S. Pags-Berhouet, F. Doz, L. Desjardins, J. Couturier, M. Gauthier-Villars, D. Stoppa-Lyonnet, C. Houdayer. Institut Curie, Paris, France.

Gene rearrangement tests are of special importance for the molecular diagnosis of diseases caused by either deletion or duplication of a specific DNA region. One example of disease caused by such gene deletions is retinoblastoma, a malignant tumor of retina that arises predominantly in children. Germ-line mutations of the *RB1* gene are associated with a predisposition to retinoblastoma. It has been demonstrated that an average 20% of the mutations found in *RB1* are large deletions of the gene. FISH or quantitative multiplex fluorescent PCR (e.g. QMPSF) are currently used to identify gene rearrangements in *RB1*. These methods are easy to use but remain costly. Moreover it is of good practice to confirm gene mutations by two different methods in routine molecular diagnosis. Therefore we evaluated the performance of a fluorescent DHPLC system for gene deletion analysis. DNAs from 5 normal controls and from 5 retinoblastoma patients with complete deletion of the gene were extracted using a perchlorate/chloroform procedure. DNA quantity was determined using a picogreen assay (Interchim) and a Genios microplate reader (Tecan), then adjusted to 25ng/l working solutions. Exons 25 and 26 of *RB1* were PCR-amplified using 50ng of DNA and standard cycling condition, except for cycle number which was set to 22. PCR products were then injected on a 3500 Wave system using non denaturing conditions and an elution gradient as predicted by WaveNavigator software, and following fluorescent detection with High Sensitivity Detection system using a double strand staining solution (Transgenomic). All samples were matched and deletions were unambiguously detected by an approximately two-fold decrease in peak area and intensity. Samples were amplified and analyzed 2 times with identical results. These preliminary results showed that a fluorescent DHPLC system could be used for deletion screening and probably allele quantification. However, it must now be tested and validated on large series to draw definite conclusions.

**Analysis of the Birt-Hogg-Dubé (BHD) tumour suppressor gene in sporadic renal cell carcinoma and colorectal cancer.** *N.F. da Silva<sup>1</sup>, D. Gentle<sup>1</sup>, L.B. Hesson<sup>2</sup>, D.G. Morton<sup>3</sup>, F. Latif<sup>1, 2</sup>, E.R. Maher<sup>1, 2</sup>.* 1) Cancer Research UK Renal Molecular Oncology Research Group, University of Birmingham, The Medical School, Edgbaston, Birmingham, B15 2TT, U.K; 2) Section of Medical and Molecular Genetics, Department of Paediatrics and Child Health, University of Birmingham, The Medical School, Edgbaston, Birmingham, B15 2TT, U.K; 3) Department of Surgery, University of Birmingham, Birmingham B15 2TT, UK.

Germline mutations in the BHD gene cause the dominantly inherited cancer susceptibility disorder, Birt-Hogg-Dubé (BHD) syndrome. Individuals with BHD are reported to have an increased risk of renal cell carcinoma and colorectal polyps and cancer. The BHD gene maps to 17p11.2 and to investigate whether somatic inactivation of the BHD gene region is implicated in the pathogenesis of sporadic RCC and colorectal cancer, we performed mutation analysis in 30 RCC primary tumours and cell lines and 35 colorectal cancers and cell lines. A somatic missense mutation (A444S) with loss of the wild type allele (consistent with Knudson's two-hit hypothesis) was detected in a primary clear cell RCC and a further missense mutation (A238V) was identified in clear cell RCC cell line for which matched normal DNA was not available. A somatic missense substitution (R392G) was identified in a primary colorectal cancer and the same change was detected in 3/6 oncocytomas tested for which matched normal DNA was not available. A germline R320Q missense variant detected in a primary colorectal cancer was not detected in 40 control individuals. However AA homozygotes for an intronic SNP (c.1517+6 G>A) were under-represented in familial cases compared to controls ( $p=0.03$ ). For some TSGs, epigenetic silencing is a more common mechanism of inactivation. No evidence of epigenetic silencing of BHD was detected in 19 colorectal cancer and RCC cell lines, and BHD promoter region hypermethylation was not detected in 20 primary RCC. These findings suggest that BHD inactivation occurs in a subset of clear cell RCC and colorectal cancer.

**Screening for genomic rearrangements of the mismatch repair genes must be included in the routine diagnosis of HNPCC.** *F. Di Fiore*<sup>1</sup>, *F. Charbonnier*<sup>1</sup>, *C. Martin*<sup>1</sup>, *S. Frerot*<sup>1</sup>, *S. Olschwang*<sup>2</sup>, *Q. Wang*<sup>3</sup>, *C. Boisson*<sup>2</sup>, *M.P. Buisine*<sup>4</sup>, *M. Nilbert*<sup>5</sup>, *A. Lindblom*<sup>6</sup>, *T. Frebourg*<sup>1</sup>. 1) Department of Genetics and INSERM EMI 9906, CHU and Faculty of Medicine, Rouen, France; 2) INSERM U434 and Hôpital Saint Antoine, Paris, France; 3) Unité d'Oncologie Moléculaire, Centre Léon Bérard, Lyon, France; 4) Laboratoire de Biochimie et Biologie Moléculaire, CHU de Lille, Lille, France; 5) Department of Oncology, University Hospital, Lund, Sweden; 6) Department of Clinical Genetics, Karolinska Hospital, Stockholm, Sweden.

We recently showed, using QMPSF (Quantitative Multiplex PCR of Short Fluorescent Fragments) that genomic rearrangements of *MSH2* are involved in 20% of the HNPCC families fulfilling the Amsterdam (AMS) criteria, without detectable point mutations. We have now integrated the QMPSF into the routine diagnosis of HNPCC and have analyzed a total of 332 HNPCC families, without detectable point mutations and corresponding to 123 AMS+ and 209 AMS- families. We identified in 37 families, corresponding respectively to 24 AMS+ (20%) and 13 AMS- (6%) families, 21 distinct exonic rearrangements of *MSH2*, removing exon(s) 1, 1-2, 1-4, 1-6, 1-7, 1-8, 1-11, 1-15, 2, 3, 4-6, 5, 5-6, 7, 7-10, 8, 9-10, 12-13, 13-15 and two cases of duplication involving exons 9-10 and 7-8. Analysis by QMPSF of 50 Kb of genomic sequences upstream the *MSH2* transcription initiation site revealed 8 distinct 5'breakpoints. QMPSF analysis of the *MSH2* promoter region in 90 AMS+, without exonic deletion, allowed us to detect a deletion removing 1.7 kb of the promoter. Analysis of *MLH1* in 182 families led us to identify, in 6/82 AMS+ (7 %) and 3/100 AMS- (3%) families, 8 exonic rearrangements of *MLH1*, removing exon(s) 1-19, 2, 4-6, 6, 7-9, 11, 13-16 and 14. We found that the selective extinction of the MMR protein was highly predictive of a *MMR* rearrangement. We conclude that *MSH2* rearrangements are involved in at least 10% of the AMS+ families, which justifies to include their search in the routine diagnosis of HNPCC. Even if genomic rearrangements of *MLH1* appear less frequent, their presence should be considered in HNPCC patients, when the immunostaining of the tumours reveals an absence of MLH1 expression.

**Broadening the WT1 mutation associated phenotype.** *H. Druker<sup>1</sup>, R. Weksberg<sup>1,2</sup>, S. O'Neill<sup>1</sup>, D. Malkin<sup>1,3</sup>, A. Khoury<sup>4</sup>, R. Grant<sup>3</sup>.* 1) Cancer Genetics Program, The Hospital for Sick Children, Toronto, ON, Canada; 2) Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, ON, Canada; 3) Haematology/Oncology, The Hospital for Sick Children, Toronto, ON, Canada; 4) Urology, The Hospital for Sick Children, Toronto, ON, Canada.

WT1 is required for the normal development and function of the urogenital tract and gonads. Constitutional mutations (MIM#194070) are associated with familial Wilms tumour and syndromes such as Denys-Drash syndrome (DDS) characterized by nephropathy (mesangial sclerosis), genital anomalies often including ambiguous genitalia or pseudohermaphroditism in XY individuals, and often a predisposition to Wilms tumour. Incomplete DDS has been defined as nephropathy with genital anomalies or Wilms tumours (Little, 1997). We report a case of a 46,XY male with a constitutional WT1 mutation with bilateral triphasic Wilms tumour with heterologous rhabdomyomatous component involving the right kidney at 10 months of age. Present in biopsies of the right and left kidneys were areas of focal segmental cystic dysplasia, and focal parenchymal atrophy and fibrosis, although no renal dysfunction was noted. At 3 years 10 months of age, triphasic Wilms occurred in the left kidney. Our patient also had hypospadias, deficient proximal urethra, cryptorchidism and chordee, all of which have been surgically corrected. Our patient did not have mesangial sclerosis, and therefore did not meet the classical DDS definition. Constitutional DNA was obtained from this patient which revealed a de novo truncating mutation R362X in exon 8 of the WT1 gene. This mutation has been reported in patients with variable expressivity from the classical DDS phenotype to a normal phenotype. Twelve patients with this mutation have been reviewed in the literature to date (Little, 1993; Kaplinsky, 1996; Heathcott, 2002). However, our patient was reported to have cystic dysplasia, a deficient proximal urethra and chordee, which are findings not yet described in patients with this mutation. This patient extends the range and variation of phenotypes that may arise from a specific germline mutation in WT1.

**Conversion technology increases the sensitivity of genetic testing in hereditary non-polyposis colorectal cancer.**

*G. Casey*<sup>1</sup>, *N. Lindor*<sup>2</sup>, *N. Papadopoulos*<sup>3</sup>, *J. Moskowitz*<sup>3</sup>, *R. Jenkins*<sup>2</sup>, *S. Thibodeau*<sup>2</sup>, *S. Gallinger*<sup>4</sup>, *B. Bapat*<sup>4</sup>, *J. Hopper*<sup>5</sup>, *J. Jass*<sup>5</sup>, *J. Young*<sup>5</sup>, *C. Buzin*<sup>7</sup>, *W. Song*<sup>7</sup>, *J. Baron*<sup>6</sup>, *S. Steelman*<sup>3</sup>, *C. Wen-Fong*<sup>7</sup>, *B. Estaki*<sup>7</sup>, *S. Sommer*<sup>7</sup>, *D. Seminara*<sup>8</sup>, *R. Haile*<sup>9</sup>. 1) Cleveland Clinic Foundation, Cleveland, OH; 2) Mayo Foundation, Rochester, MN; 3) GMP Genetics, Waltham, MA; 4) MT Sinai Hospital, Univ of Toronto, Canada; 5) University of Melbourne, Victoria, Australia; 6) Evergreen Center, Lebanon, NH; 7) City of Hope, Duarte, CA; 8) NCI, Bethesda, MD; 9) USC, LA, CA.

Background: Identification of germline mutations is a challenge. In Hereditary Non-Polyposis Colorectal Cancer (HNPCC), the mutation spectrum of MLH1 and MSH2 genes is diverse. Large deletions or promoter mutations and mutations close to splice-sites may be missed by genomic sequencing or be difficult to interpret. Methods: 93 individuals were selected from the Cooperative Family Registry for Colon Cancer Studies. Each had a colorectal cancer with high level of microsatellite instability, 84 were from families meeting the classical Amsterdam I criteria for HNPCC. From EBV transformed cell lines, the chromosomes containing alleles for MLH1 and MSH2 genes were separated in somatic cell hybrids by Conversion Technology followed by analysis of cDNA expression and cDNA sequencing of MLH1 and MSH2. Results were compared to data obtained from traditional mutation analysis of genomic DNA by sequencing of all exons and splice-sites of MLH1 and MSH2. Results: All 39 exon mutations were identified by both conversion technology and exon scanning methods. However, conversion technology was also able to identify 13 large genomic deletions, one exon duplication and 2 loss of expression mutations not identified by exon scanning. Conversion technology was also able to confirm that 16 of 20 splice site mutations resulted in altered splicing. Conclusions: Mutation analysis on haploid templates increases the sensitivity of genetic testing. Acknowledgements to the Cooperative Family Registry for Colon Cancer Studies. This study was supported in part by NCI award U01CA74799.

**Total murine mitochondrial genome screening of cutaneous tumors.** *A. Eshaghian, J.E. Sligh.* Division of Dermatology and Department of Cell and Developmental Biology, Vanderbilt University Medical Center and VA Tennessee Valley Health Care System, Nashville, TN.

The mouse mitochondrial genome is a 16,295 bp circular DNA molecule encoding 13 polypeptides involved in oxidative phosphorylation, two rRNAs, and 22 tRNAs. The accumulation of homoplasmic mitochondrial DNA (mtDNA) point mutations has been observed in various malignancies in humans. We sought to identify the potential role of mtDNA point mutations in the pathogenesis of cutaneous tumors in an animal model. We isolated total genomic DNA from ultraviolet-induced cutaneous tumors as well as from minimally-exposed skin on the ventral surface of hairless mice. The entire mouse mitochondrial genome was PCR-amplified with high-fidelity Optimase polymerase (Transgenomic) using 51 primers pairs whose products are 400-500 bp in length each with a minimum of 15% overlap. The PCR products amplified from tumor DNA were then mixed with PCR products amplified from non-tumor DNA. Denaturation of these DNAs followed by slow cool down allows for wild type DNA to re-anneal with potentially mutated DNA (tumor), forming heteroduplexes. Heteroduplexes were detected using either the WAVE denaturing high performance liquid chromatography (DHPLC) system (Transgenomic) or the Reveal temperature gradient capillary electrophoresis (TGCE) system (SpectruMedix). In an initial screen, we have identified a heteroplasmic mutation (A8875G) in the mtCOX3 gene affecting codon 90 (E90G). We have also designed a multiplex system in which multiple larger PCR products are simultaneously amplified in the same tube. The products are then digested with a restriction enzyme forming multiple smaller fragments, whose sizes vary sufficiently such that all fragments in each tube can be screened simultaneously using TGCE. This multiplex method allows for high-throughput screening of the entire mitochondrial genome for SNPs conveniently in eight tubes. We found that TGCE is as sensitive as DHPLC in mutation detection and we are screening more tumors using these methods.

**Prevalence results for five recurrent *BRCA1* rearrangement mutations in 7570 analyses.** *B. Hendrickson, A.M. Deffenbaugh, C.A. Gaglio, T. Judkins, S. Chen, B.E. Ward, T. Scholl.* Myriad Genetic Laboratories, Salt Lake City, UT.

Using an automated high-throughput, multiplexed assay, 7570 patients at risk for hereditary breast and ovarian cancer were tested for five recurrent *BRCA1* rearrangement mutations as part of the comprehensive analysis of the *BRCA1* and *BRCA2* genes. The rearrangements detected in this panel include previously characterized deletions of exons 8-9, 13, 14-20, 22 and a duplication of exon 13. From a total of 1036 patients identified with deleterious mutations, 31 (3.0%) were positive for one of these rearrangement mutations. The exon 13 duplication was the most common rearrangement and was identified in 26 patients, representing 83.9% of the rearrangement mutations and 2.5% of the total mutations. The frequency of this mutation makes this the most prevalent non-Ashkenazi *BRCA1* mutation in our test population. Also, two patients were positive for the deletion of exons 14-20 and three had the deletion of exon 22. The deletion of exon 13 and the deletion of exons 8-9 did not occur in this sample set. Over 90% of the patients with the exon 13 duplication claimed European ancestry. All instances of the exon 22 deletion and the deletion of exons 14-20 occurred in individuals of Western or Northern European ancestry.

A review of SNP haplotypes obtained from the sequencing data revealed that the exon 13 duplication and deletions of exon 14-20 and 22 reside on the consensus allele. Interestingly, because the 14-20 deletion occurs in a region that contains SNPs used to define these haplotypes, its presence can often be inferred from sequencing data when the mutant allele occurs in a context with common non-consensus alleles. This circumstance allowed us to identify four additional occurrences of this mutation.

This assay has proven to be a highly effective method to detect rearrangement mutations and improves the sensitivity of clinical genetic tests.

**Comprehensive screening for germ-line *RBI* mutations by DHPLC and QMPSF.** *S. Pagès-Berhouet, A. Laugé, C. Dehainault, V. Caux-Moncoutier, P. Karczynski, F. Doz, L. Desjardins, J. Couturier, M. Gauthier-Villars, C. Houdayer, D. Stoppa-Lyonnet.* Institut Curie, Paris, France.

Germ-line mutations of the *RBI* gene are associated with a predisposition to retinoblastoma. It is essential to identify these mutations in order to provide appropriate genetic counseling in retinoblastoma patients, but this represents an extremely challenging task, as the vast majority of mutations are unique and spread over the entire coding sequence. Since 2001, we have implemented *RBI* testing on a routine basis as part of the clinical management of retinoblastoma. As most screening techniques do not meet the requirements for efficient *RBI* testing, we have devised a semi-automated DHPLC (Denaturing High-Performance Liquid Chromatography) method for point mutation detection combined with a QMPSF (Quantitative Multiplex PCR of Short fluorescent Fragments) approach to screen for gene rearrangements. We report the results of this comprehensive screening of all exons and promoter of *RBI* in 192 unrelated patients, mostly of French origin. Among 102 bilateral and/or familial cases and 90 unilateral sporadic probands, mutations were identified in 83 (81.5%) and 5 (5.5%) cases, respectively. One half of these mutations have not been previously reported. The mutational spectrum was found to be significantly different from previous published series, displaying an astonishing amount of splice mutations and large deletions. This study demonstrates the reliability of DHPLC for *RBI* analysis, but also illustrates the need for a deletion scanning approach and the successful adaptation of QMPSF to *RBI*. Finally, considering the benefits to retinoblastoma patients, *RBI* testing should be widely implemented in routine healthcare since our study clearly illustrates its feasibility.

**A simple DHPLC-based method for quantitative analysis of allele-specific expression of the *MLH1* gene.** *M. Tosi<sup>1</sup>, G. Raux<sup>1</sup>, I. Tournier<sup>1</sup>, F. Di Fiore<sup>1</sup>, C. Martin<sup>1</sup>, I. Maréchal<sup>2</sup>, C. Leclerc<sup>2</sup>, T. Frébourg<sup>1</sup>.* 1) INSERM EMI 9906, Faculty of Medicine, Rouen, France; 2) EFS de Normandie, Rouen, France.

Quantitative measurements of allele-specific gene expression open the way to indirect detection of mutations within regulatory elements or in deep intronic regions that may result in significant physiological or pathological changes of gene expression, but can hardly be detected by the current methods of genetic analysis. Since most methods for the analysis of allele-specific gene expression are either imprecise or expensive, we have devised a simple method, based on RT-PCR and single nucleotide primer extension (SNuPE) using unlabelled dideoxy-nucleotides. Extension products were separated on a 3.0 x 75mm Helix DNA column (Varian). As a first test of this method, we genotyped 99 French unrelated control individuals for the frequent I219V polymorphism in exon 8 of *MLH1* and found 40 heterozygous subjects. We then measured the allele-specific *MLH1* expression in these heterozygous controls, using ARNs extracted from fresh blood samples taken under strictly identical conditions. Triplicates of PCR and primer extension performed on each of these 40 heterozygotes showed that this method produces allele-specific UV absorbance peaks that provide reliable measurements of allele-specific expression. No significant deviation from the expected 50% contribution of each allele was found in these controls (standard deviation 3.5%, including biological variability and measurement error). We then measured the allele-specific gene expression in 10 hereditary nonpolyposis colorectal cancer patients with different stop or frame-shift mutations or in-frame microdeletions, in order to determine which of these defects induce nonsense-mediated mRNA decay (NMD). Comparison of the allelic expression levels in these 10 patient samples with the normal range defined above allowed us to identify unambiguously the defects that induce mRNA instability, with results consistent with the current models of NMD. This study provides a sensitive tool to identify indirectly any type of *MLH1* defect that may escape detection in genomic DNA screenings but results in a quantitative change at the mRNA level.

**Molecular analysis of familial cases of renal cell cancer.** *D. Bodmer, A. Bonn , M. Eleveld, F. van Erp, E. Schoenmakers, A. Geurts van Kessel.* Department of Human Genetics, University Hospital Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.

To date, eight families have been described in literature in which constitutional chromosome 3 translocations co-segregate with a predisposition to the development of renal cell cancer (RCC). Through a multi-center study we have identified several additional families with RCC and chromosome 3 translocations. Previously, we found that loss of the derivative chromosome carrying the 3p arm and subsequent VHL mutations constitute critical steps in the development and progression of these RCCs. In addition, we hypothesized that genes located at or near the translocation breakpoints may be related to tumor initiation (Bodmer et al., 2002). In order to test this hypothesis, we have set out to positionally clone the translocation breakpoints and to characterize them in detail. Using this approach we identified two novel breakpoint-spanning genes in one of our RCC families with a t(2;3)(q35;q21) translocation. The breakpoint-spanning gene on chromosome 3, named DIRC2, encodes a putative membrane-spanning protein belonging to the major facilitator super family (MFS) of transporters. The gene disrupted by the breakpoint on chromosome 2, named DIRC3, forms fusion transcripts with HSPBAP1, a JmjC-Hsp domain gene that maps proximal to the breakpoint on chromosome 3. Currently, we are characterizing these (fusion) genes in detail at the cell biological and functional level. Together with known and novel genes to be identified by others, and us, these studies might eventually lead to a unified model for hereditary RCC development in families with constitutional chromosome 3 translocations. Bodmer et al., Human Molecular Genetics 11: 2489-2498 (2002).

**Gene expression based classification of malignant gliomas provides more accurate prediction of survival than histologic grading.** *W.A. Freije, F.E. Castro-Vargas, Z. Fang, S. Horvath, P. Mischel, L. Liau, T. Cloughesy, S. Nelson.* Human Genetics, UCLA, Los Angeles, CA.

Although histologic grading of malignant gliomas correlates strongly with survival, it is subjective and applied differently by different pathologists creating variability between treatment centers in diagnostic categories. Histology provides little insight into the underlying biology of gliomas. Large scale gene expression studies provide an attractive alternative to tumor classification schemes that can be applied uniformly and provide robust predictors. We have performed genome-wide analysis of gene expression of malignant gliomas to identify groupings of tumors which are divided into distinct prognostic groups. 59 glioblastoma multiforme(GBM), 2 grade II astrocytoma, 9 anaplastic astrocytoma, 8 grade II oligodendroglioma, 11 grade III oligodendroglioma, and 9 mixed grade III oligo-astrocytoma samples (total N=98) were analyzed for approximately 31,000 different transcripts using the Affymetrix U133A/B microarrays. Using a partial least squares supervised dimension reduction technique, 8 linear components were defined which accounted for 96.7% of the power to predict survival. Using these 8 components the unsupervised classification model of partition around the medoids was used to classify the glioma samples. This yielded two groups, GBM-like and NonGBM-like. The GBM-like group contained 51 GBM, 5 oligodendroglioma, and 1 mixed oligo-astrocytoma samples. The NonGBM-like group contained 8 GBM, 11 astrocytoma, 14 oligodendroglioma, and 8 mixed astro-oligodendroglioma samples. Kaplan-Meier survival analysis of these two groups were significantly different, and significantly more predictive than histologic grading, with a 50% survival time of approximately 250 days for the GBM-like group, and greater than 2500 days for the NonGBM-like group ( $P=3.3e-11$ ). To test the robustness of the model, the results were validated on an independent set of malignant glioma data which used the U95Av2 arrays( $p=0.00013$ ) (Nutt et al, Cancer Research 2003 63:1602-7). Such classification schemes may be used in the future to supplant the current system of prognostication through histopathology.

**Synergistic effects of Aurora-A and p53 in liver regeneration and pathogenesis.** *H.Y. Chu*<sup>1</sup>, *H.J. Chen*<sup>2</sup>, *Y.R. Chen*<sup>3</sup>, *C.K. Chou*<sup>2,3,5</sup>, *C.W. Chi*<sup>4,5</sup>, *T.F. Tsai*<sup>1,2,3,5</sup>. 1) Institute of Genetics, National Yang-Ming University, Taipei, Taiwan; 2) Division of Molecular and Genomic Medicine, National Health Research Institute, Taipei, Taiwan; 3) Department of Life Science, National Yang-Ming University, Taipei, Taiwan; 4) Institute of Pharmacology, Natl Yang-Ming Univ, Taipei, Taiwan; 5) Department of Medical Research and Education, Veterans General Hospital, Taipei, Taiwan.

Hepatocellular carcinoma (HCC) is one of the most common forms of human cancers. The formation of HCC is a multi-step process involving the occurrence of somatic mutations, loss of tumor suppressor gene(s), and activation of oncogenes. Our goal is to establish an *in vivo* mouse model of HCC. Aurora-A has been suggested as an oncogene in cultured cells, and overexpression of Aurora-A has been found in HCC. We have successfully developed transgenic lines with human Aurora-A cDNA over-expressed from the PEPCK promoter. The effect of Aurora-A overexpression on cell cycle progression was analyzed in liver regeneration induced by two-third partial hepatectomy (PHx). Abnormal cell cycle progression was detected based on expression profile of the cell cycle marker, BrdU labeling and mitotic index. Prolonged S phase and decreased mitotic figures were observed in the regenerated livers of the Aurora-A transgenic mice. Several foci of cell death in transgenic livers were detected at day 1, 3, 4, and 7 after PHx. The pathological abnormality may due to arresting of G2/M progression. The data also suggest a negative regulation in the Aurora-A transgenic livers under physiological condition. Previous report indicated that Aurora-A directly interact with p53 in cell culture system. It was also suggested that overexpression of Aurora-A in p53 knockout (p53<sup>-/-</sup>) mouse embryonic fibroblasts (MEF) favor centrosome amplification. To delineate their roles in relationship to cell cycle progression and pathogenesis, the Aurora-A transgenic mice with p53 knockout (p53<sup>-/-</sup>) background were studied. Our data suggest that there are synergistic effects of Aurora-A deregulation and p53 deficiency in liver regeneration and carcinogenesis process.

**Analysis of *CHEK2* for ovarian cancer susceptibility.** *B.E. Baysal*<sup>1</sup>, *J.A. DeLoia*<sup>1</sup>, *M.T. Goodman*<sup>2</sup>, *J.E. Willett-Brozick*<sup>1</sup>, *P. Watson*<sup>3</sup>, *M.J. Birrer*<sup>4</sup>, *H.H. Gallion*<sup>1</sup>. 1) Ob/Gyn&Reproductive Sci., Univ. Pittsburgh Sch. of Med., Pittsburgh, PA; 2) Cancer Res. Ctr. of Hawaii, Univ. Hawaii, Honolulu, HI; 3) Creighton Univ., Omaha, NE; 4) Natl. Cancer Inst. (NCI) Mol. Mech. Section, Rockville, MD.

Ovarian cancer (OvCa), with a lifetime incidence of approximately 1%, is the fifth leading cause of death from cancer among women. Familial predisposition has been observed in up to 20% of the OvCa cases. It has been estimated that up to 10% of all OvCas result from an inherited mutation in the *BRCA1* or *BRCA2* genes. However, the genetic factors that may contribute to the etiology in up to 50% of the familial and in the nonfamilial cases are largely unknown. Recently, germline mutations in *CHEK2* have been identified as a low-penetrance risk factor for familial breast cancer. However, the role of *CHEK2* in OvCa is unknown. Because of the commonality of genetic risk factors between breast and OvCas, we are investigating the role of *CHEK2* for OvCa susceptibility in a large number of subjects ascertained throughout the United States.

The subjects were recruited through the Gynecologic Oncology Group (GOG) protocols 143, 144, 172, 182; the University of Hawaii Cancer Res. Ctr.; and Creighton University. GOG is a premier NCI Sponsored Cancer Cooperative Clinical Trials Research Group. First, we have tested a common deletion variant, del1100C that has been observed in approximately 1% of the general population, by Pyrosequencing and found this deletion in only 2 of 345 (~0.6%) unselected OvCa subjects. Both deletion carriers were from a subgroup with a positive family history for breast or OvCas but no *BRCA1* mutations (n=40). Initial sequencing of the fourteen exons of the *CHEK2* gene in 15 subjects with a positive family history did not identify further mutations, only a previously identified silent variant (E84E). These findings suggest that the *CHEK2* gene del1100C variant does not play a role in predisposition to sporadic OvCa, but may be involved in familial OvCa. The rarity of the deletion variant in the patient population may limit its practical value in the clinical management of OvCa patients.

**Transcriptional deregulation by adenovirus E1A in human tumor cells: reprogramming of tumor cells to less malignant phenotypes.** *J. Dorsman*<sup>1, 4</sup>, *A. Teunisse*<sup>2</sup>, *B. Pepers*<sup>1</sup>, *T. van Laar*<sup>5</sup>, *G. van Ommen*<sup>1</sup>, *A. Zantema*<sup>2</sup>, *A. van der Eb*<sup>3</sup>. 1) HCG, LUMC, Leiden, Netherlands; 2) MCB1, LUMC, Leiden, Netherlands; 3) TG, LUMC, Leiden, Netherlands; 4) O&G, VUmc, Amsterdam, Netherlands; 5) MC, NKI, Amsterdam, Netherlands.

The adenovirus 5 (Ad5) E1A protein can reverse the transformed phenotype in a wide range of human tumor cells and can suppress primary human tumor growth. E1A apparently interferes with oncogenic pathways by its ability to reprogram transcription in human tumor cells. To get more insight which genes are deregulated by E1A in human tumor cells, Affymetrix expression array studies were performed analysing two different model systems. Firstly, human osteosarcoma U-2 OS cells were transfected with constructs expressing the 12S Ad5 E1A protein, in parallel with the transfection of a control vector. Two independent experiments showed the down-regulation of various genes involved in stress responses including, GADD45, GADD153 and ATF3. In agreement with previous studies, an up-regulation of genes involved in the heat shock response was observed. The Affymetrix array data were validated by Northern blot. To study the long term effects of E1A, glioblastoma T98G cell lines which stably express the 12S Ad5 E1A gene were compared with cell lines generated by transfection of the same vector lacking the E1A gene. The E1A-expressing cell lines do not give rise to any colony formation in soft agar, whereas the control cell lines efficiently form colonies under the same conditions. Also in these stable cell lines the expression of genes involved in the heat shock response are up-regulated, including HSP40 and HSP110. In addition, other groups of co-regulated genes are found, including the down-regulation of a group of genes coding for metalloproteases and of genes involved in xenobiotics metabolism. The implications of these findings are discussed.

**Transcription Factor Profiling of Endometrial Cancers.** *I. DASKALAKIS<sup>1</sup>, N. SACCONI<sup>1</sup>, D. MUTCH<sup>2</sup>, P. GOODFELLOW<sup>2</sup>, M. LOVETT<sup>1</sup>, A.M. BOWCOCK<sup>1</sup>.* 1) DEPARTMENT OF GENETICS, WASHINGTON UNIVERSITY, ST. LOUIS, MO; 2) DEPARTMENT OF SURGERY, WASHINGTON UNIVERSITY, ST. LOUIS, MO.

Endometrial cancer is the most common form of pelvic malignancy representing 7-10% of all invasive cancers in women. One molecular classification of endometrial cancers is based on the presence of Microsatellite Instability (MSI). MSI- tumors are associated with major chromosomal instability or aneuploidy while MSI+ tumors harbor alterations in the length of short repeat tracts and an increase in mutation rate. Approximately 25% of endometrial cancers are MSI+. In some cases, MSI+ tumors are due to mutations in mismatch repair genes eg. MSH2, MLH1 and MSH6. The objectives of this study are to understand the molecular basis of endometrial cancer and to identify the tumorigenic targets in the MSI+ tumors. Gene expression profiles of four well characterized MSI+ and MSI tumor samples, matched on the basis of age and stage were compared by hybridization to a unique custom microarray comprised of 1700 transcription factors (Tfs). Statistical validation of the results was performed using the method of Wolfinger et al. 9 Tfs were downregulated in MSI+ tumors ( $p < 0.0001$ ). At a cut-off p value of 0.05, 30 Tfs were downregulated MSI+ tumors. Many of the altered Tfs previously shown to be involved in tumor formation were downregulated in the MSI- tumors and included p53 and MYCL1. However, a number of novel Tfs were also down-regulated in MSI- tumors and included ZNF73 and KIAA0478. TaqMan RT PCR is being used to confirm these findings in larger numbers of samples. 9 Tfs downregulated in MSI- tumors and mapping to tumor suppressor loci are being mutationally analyzed with denaturing high performance liquid chromatography. The ability to interrogate large numbers of tumors with a comprehensive set of transcription factors is potentially a valuable resource for the identification of novel tumor suppressors.

**Complementary analysis of clear cell renal carcinoma by array-based CGH and expression profiling:**

**Identification of relevant genomic aberrations.** *E. Lausch*<sup>1</sup>, *B. Fritz*<sup>2</sup>, *D. Reutzel*<sup>1</sup>, *B. Radlwimmer*<sup>2</sup>, *C. Spangenberg*<sup>1</sup>, *W. Brenner*<sup>3</sup>, *H. Decker*<sup>4</sup>, *P. Lichter*<sup>2</sup>, *B. Zabel*<sup>1</sup>. 1) Children's Hospital, Mainz; 2) DKFZ, Heidelberg; 3) Department of Urology, Mainz; 4) Bioscientia Institut, Ingelheim.

Renal cell carcinoma (RCC) is the most common type of renal cancer accounting for 3% of all malignancies in men. The clinical manifestation of RCC is highly variable. More than 1/3 of patients with RCC present with metastatic disease at the time of diagnosis, an additional 20% develop metastasis within 5 years after nephrectomy. The prognosis of patients with metastatic disease remains poor. Tumor biology is partly related to the histopathological subtypes of RCC which in turn are associated with a broad spectrum of genetic alterations and morphological signs of differentiation. The most common subtype, clear cell RCC is strongly associated with somatic deletions of the short arm of chromosome 3 coupled with inactivation of the *VHL* gene. Other common chromosomal alterations in progressing clear cell RCC include deletions of 6q, 8p, 9, and 14q, and duplication of 5q. Although some of these genomic aberrations appear to be linked to clinical outcome, specific genes have not yet been identified. In order to understand the underlying molecular mechanisms causing different biological behaviour of RCC we performed a parallel analysis of 19 clear cell RCC specimens and patient-matched normal tissue by array-based comparative genomic hybridization and expression profiling. The genomic microarray contained 2300 BAC elements allowing to detect copy number changes with an average resolution of 1.5 Mbp. Expression profiling was carried out using microarrays consisting of 3600 nonredundant sequence-validated human cDNAs selected on their putative involvement in tumorigenesis. Differentially expressed genes were confirmed by reverse quantitative PCR. Classification of tumors based on their genomic and expression profile was achieved by cluster analysis. Profiles were used to stratify tumors according to clinical behaviour. Congruent changes in genomic loci and gene expression were correlated and are the basis for further functional analysis. Candidate 'progression suppressor' genes will be discussed.

**Genome-wide analysis of genetic alterations in tumor cell lines by Ccap BAC microarray.** *H. He<sup>1</sup>, A. Roschke<sup>1</sup>, R. Yonescu<sup>1</sup>, E. Hatchwell<sup>2</sup>, T. Ried<sup>1</sup>, I. Kirsch<sup>1</sup>.* 1) Genetic Branch, CCR, NCI, NIH Bethesda, MD; 2) Genome Research Center, Cold Spring Harbor Laboratory, Woodbury, NY.

Recently, the first Ccap BAC microarray has been developed and printed by the NCI and the Cold Spring Harbor Laboratory. This array contains over 1,300 Ccap BAC clones and all of these BAC clones have been mapped by high-resolution fluorescence-in-situ-hybridization (FISH) to single sites on chromosomes. The Ccap BAC set is also physically mapped and can be localized by alignment of BAC end sequences to the finished HGP contig. Ccap BAC clones are localized at 12-Mb intervals across the entire genome. Information about those clones is available on a publicly accessible web site <http://www.ncbi.nlm.nih.gov/CCAP> BAC clones were amplified by DOP-PCR and printed onto glass slides. Using this Ccap BAC array, several tumor cell lines have been analyzed. The CGH microarray data have been validated by chromosomal CGH and SKY on the same cell lines. Copy number changes of small regions (microdeletions and duplications) were confirmed by interphase FISH. Our data demonstrated that Ccap BAC arrays were able to define copy number changes of whole chromosome or chromosome regions in cancer cell lines. The Ccap BAC array provides a valuable tool, which not only allows definition of a target area of gain or loss from cancer samples, but also leads seamlessly from a cytogenetic band to the primary DNA sequence allowing integration of molecular cytogenetics and genomics.

**Recombinant Protein encoded by Esophageal Cancer Related Gene-1 (ECRG-1) inhibits the proliferation of the Esophageal carcinoma cell line *in vitro*.** *Y. Wang*<sup>1, 2</sup>, *H. Tang*<sup>2</sup>, *S. Lu*<sup>2</sup>, *T.-J. Chen*<sup>1</sup>. 1) Dept Medical Genetics, Univ South Alabama, Mobile, AL; 2) Cancer Institute (Hospital), Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100021.

The ECRG-1 gene was isolated and characterized by our group from the differential display studies of esophageal cancer and normal esophageal epithelia cells. The ECRG-1 gene is expressed in several human tissues, including the normal esophageal epithelia cells. The expression of ECRG-1 gene is down regulated in esophageal cancer tissue. To study the function of this gene on the esophageal carcinoma cells (NEC), the ECRG-1 cDNA was cloned and over-expressed as a fusion protein with GST. The recombinant protein was purified and confirmed with Western blot. Different concentrations of ECRG-1 protein were incubated with NEC cells. The cell numbers of NEC were determined by the MTT method. The results shown that the proliferation of the NEC cells was inhibited by the ECRG-1 protein starting at a concentration of 50g/ml. With increasing the ECRG-1 protein concentration, the inhibition rate increased. After incubation with 400g/ml ECRG-1protein for 24, 48, 72, and 96 hours, the inhibition rates were 12%, 60%, 62% and 65%, respectively. These inhibition rates were significantly different from those on the control groups ( $P < 0.001$ ), including GST protein alone. Cell cycle analysis using flow cytometry indicated that the percentage of NEC cells on G2/M phase increased from less than 15% on control groups to 30% on ECRG-1 protein treated NEC cells. These results suggested that the ECRG-1 gene might play an important role on the control of esophageal carcinoma proliferation. The possible inhibition mechanism may involve the regulation of cell cycle, particularly on the G2/M phase checkpoints.

**Identification and pathway mapping of brain tumor progression associated genes.** *C. Tso<sup>1</sup>, R. Shai<sup>1</sup>, W. Freije<sup>1</sup>, E. Castro<sup>1</sup>, K. Mitsouras<sup>1</sup>, Z. Chen<sup>1</sup>, B. Merriman<sup>1</sup>, L. Liau<sup>3</sup>, P. Mischel<sup>2</sup>, S. Horvath<sup>1</sup>, S. Nelson<sup>1</sup>.* 1) Human Genetics, UCLA, Los Angeles, CA; 2) Pathology, UCLA, Los Angeles, CA; 3) Surgery, UCLA, Los Angeles, CA.

In order to elucidate the molecular mechanisms underlying brain tumor progression, we have established an oligonucleotide microarray-based transcription database for malignant brain tumors with defined pathological grading, as well as survival and treatment data. The database contains over 200 individual tumors as well as 15 normal brain tissue samples. We have identified 67 genes which have a likely oncogenic effect due to overexpression and 33 whose effect is mediated by loss of expression. These include genes which encode proteins involved in cell cycle/growth, signaling, apoptosis, metabolism, structure, motility, RNA processing, transport and traffic, angiogenesis and DNA and protein repair. A subset of these 100 genes have been screened in unrelated types of high-grade tumor cell lines, derived from cancers of the prostate, bladder, kidney, lung, and breast as well as melanoma. Most of the genes tested were also highly expressed in these cell lines, indicating an existing common pathway(s) operating in most high-grade tumors. In order to elucidate the pathway link of our identified high-grade tumor associated factors, we have compared the gene expression levels in U87 GBM cell line treated with and without inhibitors for PI3 kinase (Ly294002), MEK1/2 (U0126), EGFR (AG1478), and IGFR (AG1024), respectively or combined. Among 26 genes screened, 16 genes are altered (2-10 fold changes) by at least one of these treatments. Based on the gene regulation profile, a pathologic network is proposed.

**Molecular analyses examining the consequences of reversible ERBB2-expression in breast carcinoma cells. C.**

*Spangenberg*<sup>1</sup>, *T. Trost*<sup>1</sup>, *E. Lausch*<sup>1</sup>, *S. Fees*<sup>1</sup>, *D. Prawitt*<sup>1</sup>, *J.G. Hengstler*<sup>2</sup>, *U. Sahin*<sup>3</sup>, *O. Türeci*<sup>3</sup>, *B. Zabel*<sup>1</sup>. 1) Children's Hospital, Univ Mainz, Germany; 2) Institute of Toxicology, Univ Mainz, Germany; 3) Department of Medicine III, Univ Mainz, Germany.

Members of the *EGFR* gene family encode type I receptor tyrosine kinases involved in the transmission of proliferative as well as differentiating signals in a variety of cells. The level of expression of the ERBB2 protooncogene is directly correlated with the prognosis of several human malignant neoplasias, like breast and ovarian carcinoma. Upregulation of ERBB2-expression which leads to aberrant signaling can frequently be attributed to genomic amplification of the *ERBB2*-locus on chromosome 17q21. A different mechanism known to cause inadequate ERBB2-activation is based on shedding large parts of the extracellular portion of the receptor. Both processes lead to ligand-independent activation of ERBB2 signaling cascades that ultimately result in the cellular changes associated with malignant transformation. To examine the cellular processes associated with ectopic ERBB2-signaling we reversibly overexpressed a mutant version of the ERBB2-protein (NeuNT; the homologous rat-protein constitutively activated by a point-mutation) in human MCF7 breast carcinoma cells using the Tetracycline regulatory system. NeuNT-expression resulted in prominent phenotypic alterations 16-48h after NeuNT-induction in several independent MCF7/Tet-NeuT clones. Initially, these morphologic changes resemble the epithel-to-mesenchyme transition that has been described for the process of carcinogenesis (cellular protrusions, spindle-morphology) whereas at later time points the cells flattened and displayed a "senescent" phenotype. When NeuNT was expressed transiently, complete reversibility of the phenotypic alterations could be observed. In order to correlate the phenotypic response with intracellular signaling pathways, inhibitors for different signal transduction cascades known to be engaged in ERBB2-signaling (PI3K/AKT, P42/P44 MAPK, JNK, P38 MAPK) were applied. Using this approach we could attribute the NeuNT-mediated phenotypic epithelial-mesenchymal transition of MCF7-cells to the activation of the p38 MAPK-pathway.

**Elucidation of ependymoma-associated oncogenic pathways by comparative SAGE analysis.** *B. Schmucker, H. Steinhart, H. Iro.* Molecular Genetics, Friedrich-Alexander-University of Erlangen-Nuremberg, Erlangen, Germany.

To gain insight into molecular basis of ependymomas, we first generated a SAGE library of normal hippocampus pooled of 19 Caucasians and then compared global gene expression profiles of 5 SAGE ependymoma libraries to control SAGE libraries from normal hippocampus and normal total brain. The sequence analysis of 16,605 tags of the hippocampus SAGE library revealed 9,829 unique transcripts corresponding to known genes, ESTs or novel transcripts. 4 of the top-10 transcripts representing the highest expression levels in hippocampus did not have any EST or GenBank entry. Comparison of the transcriptomes of the ependymomas to normal brain included more than 360,000 tags and provided a dataset of 40,387 transcripts including their abundance. To generate a profile of genes significantly overexpressed in ependymomas, the significance filter was set to  $p < 0.001$  and the expression factor to at least 4-fold in each of the ependymoma libraries. These very stringent criteria were fulfilled by 26 genes. Filamin A (overexpressed) is involved in the Rho pathway. It binds GTPases, including Rac1, cdc42, RhoA, and to the p21-activated kinase 1 (pak1). Pak1 is an effector of Rac/cdc42 implicated in proliferation and cell survival. Interestingly, pak1 was found to be significantly downregulated in ependymoma. pak1 was reported to phosphorylate histone H3 (here), which are linked to transcription activation. Filamin A participates in TNF- signal transduction by activation of the SAPK pathway and has a negative role in MAPK-mediated Elk-1 transcription in response to insulin by direct binding to the insulin receptor. We found alteration of the RAS/MAPK pathway further by: MAP2K1, MAP2K4, MAPKAPK3, MAPK8IP2. IGF signaling cascade may be involved in pathogenesis of ependymoma since: IGF2 splice form 1 and 2, SPARC, IGFBP-7. Mediators of the RAS/RHO pathway are dysregulated: MARCKS, calmodulin-3, S100-calcium binding protein-A10. So far, no data about pathways in ependymoma are available and strikingly, the identified signaling cascades are distinct from those of gliomas (p53-, RB-, PTEN-pathways and PDGF-, EGF- growth factor pathways).

**The regulatory role of THY-1 on other putative ovarian tumor suppressor genes (PTSG).** *N. Wang<sup>1</sup>, L. Li<sup>1</sup>, R. Miller<sup>2</sup>, J. Xu<sup>1</sup>, H. Abeysinghe<sup>1</sup>.* 1) Dept Pathology, Univ Rochester Sch Medicine, Rochester, NY; 2) Dept OB/GYN, Univ Rochester Sch Medicine, Rochester, NY.

We have previously reported that the introduction of chromosome 11 into ovarian cancer cell line, SKOV-3, leads to the suppression of tumorigenicity and the up-regulation of 7 chromosome 11 genes which include: CRYAB, LDHA, Thy-1, and Thy-1-co-transcribed genes and 4 non-chromosome 11 PTSG's: CAV1, HPAC3, IGFBP7, and SPARC. Also using transfection studies, we have demonstrated previously the tumor suppressing nature of Thy-1. In the present study, using cDNA microarray combined with RT-PCR, we demonstrated that 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 4 putative tumor suppressor genes: CAV1, HAAC3, IGFBP7 and CDH6 as well as CRYAB and the THY-1-co-transcribed gene. In contrast, the expression of SPARC is not affected by the abolishing of THY-1 expression which indicates a SPARC regulating gene, not Thy-1, is located on chromosome 11. In conclusion, our data suggests the crucial role of Thy-1 in ovarian tumor suppression through its regulatory effect on other putative tumor suppressor genes.

**Molecular Genetic Profiling of Normal Prostate, Benign Prostatic Hyperplasia, Dysplasia, Gleason Grade 3 and 4/5 Prostate Cancer.** Z. Zhang<sup>1</sup>, M. Mahadevappa<sup>1</sup>, MC. Caldwell<sup>2</sup>, Z. Chen<sup>2</sup>, Z. Fan<sup>2</sup>, JE. McNeal<sup>2</sup>, R. Nolley<sup>2</sup>, TA. Stamey<sup>2</sup>, JA. Palma<sup>1</sup>, WA. Warrington<sup>1</sup>. 1) Clinical Applications R&D, Affymetrix Inc., Santa Clara, CA.95051; 2) Department of Urology, Stanford University School of Medicine, Stanford, CA 94305.

Prostate cancer (PC) is one of the most common causes of cancer death among American men and is by far the most prevalent of all human malignancies with the exception of skin cancer. Serum prostate-specific antigen (PSA) between 2-10 ng/ml has been widely used in the U.S. as a marker for PC, but in this range serum PSA is largely related to benign prostatic hyperplasia (BPH), and correlates poorly with PC curative outcome. In this study we compared gene expression activity in the normal prostate, BPH, dysplasia of prostate and prostate cancer in an attempt to better understand the etiology of PC and to aid in the development of new PC serum markers and/or an array-based diagnostic. More than 80 labeled targets from prostate central zone (CZ), BPH, dysplasia, Gleason grade 3 (GG3) and Gleason grade 4/5 (GG4/5) were hybridized to high-density oligo nucleotide microarrays containing probes representing ~22,000 full-length human genes. Using a number of analysis methods, genes differentially expressed between tissues were identified and functionally annotated. Clustering using differentially expressed candidates grouped the samples distinctly. A minimal set of genes was identified for class prediction. A number of candidates were validated by QRT-PCR. The candidates include genes previously associated with PC, including racemase, as well as genes associated with oncogenesis, transcription, signal transduction and apoptosis. New candidates involved in pathways of interest were identified and will be discussed.

**Caspase 8 is Associated with Granules in Normal but not Malignant Prostate Tissue as Detected by Tissue Microarray Analysis.** *Y. Elshimali, S. Hanna, B. Bonavida, D. Chia, D. Seligson, L. Goodglick.* Department of Pathology and laboratory Medicine, and Human Genetics. David Geffen School of Medicine, UCLA. Los Angeles, CA 90095.

Caspase 8 is a cysteine protease which is important in the initiation and execution of receptor-triggered apoptosis. Caspase 8 is synthesized in an inactive, pro-protein form. However, upon induction of the apoptotic pathway, caspase 8, along with caspase 10, are recruited to the death-induced signaling complex where upon both proteins are cleaved to form active effector enzymes. Because of the central role of this and other caspases in apoptosis signaling, we have started to explore the expression of these proteins in various malignancies using tissue microarray analysis. We hypothesized that malignancies would be dysfunctional in one or more steps of the apoptotic pathway. A prostate tissue microarray was constructed consisting of 1364 tissue cores representing 246 patients. The microarray was stained with an anti-caspase 8 Ab and scored based on signal intensity, percentage of cellular staining, and intracellular localization. Both malignant and benign glandular cells demonstrated cytoplasmic caspase 8 staining. Interestingly, caspase 8 also was associated with relatively large (~0.1-3.0um) intracellular granular complexes seen in both basal and epithelial cells, predominantly in the normal cells. We have started to examine the nature of these granules, and their correlative and/or functional relationship to malignancy. First, we observed that most other proteins examined, did not colocalize to these granules. Second, granular caspase 8 staining did not co-localize with CD59 nor PSA, thus suggesting that caspase 8 was not present in the 'prostosome granules' or the prostate secretory granules (PSG), respectively. Third, caspase 8 was associated with similar granule structures in normal colonic epithelium. In conclusion, our results suggest a unique granule-association of caspase 8 in epithelium undergoing normal cellular turnover. That the granule-associated caspase 8 is decreased in prostate cancer epithelium is an intriguing observation which is currently being studied in our laboratory.

Program Nr: 505 from 2003 ASHG Annual Meeting

**A survey of receptor tyrosine kinases FLT3, c-KIT, and CSF1R polymorphisms in AML patients.** *A.A. Chen, C.M. Sullivan, N. Iartchouk, A. Parker, N.A. Giese, N.A. Lokker.* Millennium Pharmaceuticals, Cambridge, MA.

Mutations in FLT3 (fms-related tyrosine kinase, a type III receptor tyrosine kinase) have been associated with acute myeloid leukemia (AML). Specifically, an internal tandem duplication (ITD) in the juxtamembrane region, seen in 21-40% of AML patients, has been shown to constitutively activate this receptor and to associate with poor prognosis. We have sequenced DNA from the bone marrow of 18 patients to determine if there exist additional mutations in FLT3 that are predictive of prognosis. We additionally sequenced two related receptor tyrosine kinases, KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) and CSF1R (colony stimulating factor 1 receptor) to determine if there are additional activating mutations present in these genes, both of which are thought to play a role in AML. The FLT3 ITD mutation was the only non-synonymous mutation found in FLT3. There was one non-synonymous mutation found in each of the other two genes, KIT and CSF1R. Additionally, three patients appear to exhibit loss of heterozygosity at the FLT3 locus. Our data indicate polymorphisms in the FLT3, KIT, and CSF1R genes in AML blast cells, which may have utility as biomarkers for response to tyrosine kinase inhibitors.

**Polymorphisms in CYP3A5 may not be associated with levels of tamoxifen and its metabolites in breast cancer patients.** *A. Deigert, L. Lewis, K. Tkaczuk, J. Flaws.* Univ Maryland, Baltimore, MD.

Tamoxifen (TAM) is a widely used antiestrogen in women with breast cancer and in women at high risk of developing breast cancer. TAM is metabolized by the cytochrome P450 (CYP450) family of enzymes into two major metabolites, 4-hydroxytamoxifen (4-OHT) and N-desmethyltamoxifen (N-DMT). Recent studies show that CYP3A5 is a major enzyme involved in the metabolism of TAM. Although this enzyme has been shown to have two polymorphisms (CYP3A5\*3 and CYP3A5\*6), the impacts of the polymorphisms on TAM metabolism are not known. We hypothesized that polymorphic status in CYP3A5 affects the rate of metabolism of TAM to 4-OHT and N-DMT. To test this hypothesis, we recruited 63 women with breast cancer from a single clinic at the University of Maryland who had been taking TAM for at least 30 days. A survey was completed by each patient to obtain information on age, race, potential confounding factors, and adverse side effects. Blood samples were obtained from each patient and were subjected to genomic DNA extraction followed by polymerase chain reaction for CYP3A5\*3 and CYP3A5\*6. The associations between polymorphic status and TAM, 4-OHT and N-DMT levels were assessed using t-tests and logistic regression models. Our data indicate that 40.7% of the women had the CYP3A5\*3 polymorphism and 9.1% had the CYP3A5\*6 polymorphism. Our data also show that Caucasian women are 2.5 times more likely to carry the CYP3A5\*3 polymorphism than African-American women. The CYP3A5\*6 polymorphism was seen only in African-American patients. No significant differences were seen in TAM, N-DMT and 4-OHT levels by polymorphic status. For example, levels of TAM were 68.79.3ng/ml in women without CYP3A5\*3, and 73.14.7ng/ml in women with CYP3A5\*3. Levels of N-DMT were 117.429.2ng/ml in women without CYP3A5\*3, and 110.811.7ng/ml in women with CYP3A5\*3. These data suggest that two known polymorphisms in CYP3A5 (CYP3A5\*3 and CYP3A5\*6) are not associated with levels of TAM or its metabolites. However, there may be a racial distribution of CYP3A5\*3 and CYP3A5\*6 among breast cancer patients. Supported by DOD grant DAMD 17-00-1-0321 and the University of Maryland, Maryland Statewide Health Network.

**CHEK2:1100delC mutation and elevated breast cancer risk.** *L.H.M. Pereira<sup>1</sup>, A.J. Sigurdson<sup>2</sup>, M.M. Doody<sup>2</sup>, M.A. Pineda<sup>1</sup>, B.H. Alexander<sup>4</sup>, M.H. Greene<sup>3</sup>, J.P. Struewing<sup>1</sup>.* 1) Laboratory of Population Genetics; 2) Radiation Epidemiology Branch; 3) Clinical Genetics Branch, DHHS/NIH/NCI, Bethesda, MD; 4) Division of Environmental and Occupational Health, University of Minnesota, Minneapolis, MN.

The 1100delC mutation in *CHEK2*, a cell cycle checkpoint gene, was initially identified as a rare cause of Li-Fraumeni Syndrome. It has been associated with familial breast cancer, predominantly among European subjects, being present in 1 - 1.5% of controls, ~ 2% of unselected breast cancer cases, and in 3 - 5% of familial breast cancer patients. We evaluated this mutation in 2 settings: a nested breast cancer case-control study within a prospective cohort study of U.S. radiologic technologists, and in 21 probands from *BRCA1/2* mutation-negative breast/ovarian cancer families (mean of 4 cases per family). Case-control subjects included 829 prevalent breast cancer cases and 859 controls without breast cancer, frequency matched on age. The mutation was detected using a Taqman 5'-nuclease assay, and confirmed by dHPLC. The mutation was present in 9 (1.1%) cases and 4 controls (0.5%). Using logistic regression analysis, the unadjusted odds ratio was 2.3 (95% C.L. 0.7 8, P=0.16), and was unchanged after adjustment for other risk factors. The small number of carriers precluded occupational radiation risk analyses stratified on mutation status. Mutations were more common in breast cancer cases diagnosed with a second primary other than breast cancer (2.6%) and when diagnosed before age 41 (2.5%). Cases with an affected first degree relative were only slightly more likely to be mutation positive (1.2%) while controls were more than three times as likely to be positive (1.6%). Two of 21 (9.5%) probands were positive (1 proband had breast, ovarian, and thyroid primaries), and 3 of 3 affected sisters with DNA available from the families were also positive, although a daughter with osteosarcoma tested negative for the mutation. Our results suggest that the *CHEK2:1100delC* mutation may be a risk factor for both sporadic and familial breast cancer in the U.S. population. Because of its low frequency, even if it is causal, the attributable fraction of breast cancer due to the mutation is only approximately 0.6%.

**Allele frequencies of single nucleotide polymorphisms (SNPs) in 38 candidate genes for gene-environment studies on cancer: data from population-based Japanese random samples.** *K. Yoshimura*<sup>1</sup>, *T. Hanaoka*<sup>2</sup>, *S. Ohnami*<sup>3</sup>, *S. Ohnami*<sup>3</sup>, *T. Kohno*<sup>4</sup>, *Y. Liu*<sup>1</sup>, *T. Yoshida*<sup>3</sup>, *H. Sakamoto*<sup>3</sup>, *S. Tsugane*<sup>2</sup>. 1) Cancer Information and Epidemiology Division; 2) Epidemiology and Biostatistics Division; 3) Genetics Division; 4) Genetics Division, National Cancer Center Research Institute, Tokyo, JAPAN.

Knowledge of genetic polymorphisms in gene-environment studies may contribute to more accurate identification of avoidable risks, and to developing tailor-made preventative measures. The aim of this study is to describe the allele frequencies of single nucleotide polymorphisms (SNPs) of select genes, which may be included in future gene-environment studies on cancer in Japan. SNP typing was performed on middle-aged Japanese male subjects randomly selected from the general population in five areas of Japan. We genotyped and calculated allele frequencies of 152 SNPs located on 38 genes: CYP1A1, CYP1B1, CYP2C19, CYP2E1, CYP17, AHR, ESR1, ESR2, ERRRG, PGR, EPHX1, EPHX2, HSD17B2, HSD17B3, GSTM2, GSTM3, GSTT2, GSTP1, NAT1, NAT2, COMT, ADH1A, ADH1B, ADH1C, ALDH2, NOS2A, NOS3, IL1A, IL1B, OGG1, NUDT1 (MTH1), DRD2, DRD3, DRD4, SLC6A4, NR3C1 (GCCR), MTHFR, and NQO1. In the present study, the Japanese allele frequencies were verified by using nation-wide population samples.

**Identification of sequence variants in the 17-hydroxysteroid dehydrogenase type 2 gene in French-Canadian high-risk breast cancer families.** *M. Plourde<sup>1</sup>, C. Manhes<sup>1</sup>, G. Leblanc<sup>1</sup>, F. Durocher<sup>1</sup>, M. Dumont<sup>1</sup>, INHERIT BRCA<sub>s</sub> collaborators<sup>1</sup>, J. Simard<sup>1,2</sup>.* 1) Cancer Genomics Laboratory and; 2) Cancer Research Chair in Oncogenetics, CHUL Research Center, CHUQ, Laval University, Quebec, Quebec, Canada.

The 17-hydroxysteroid dehydrogenase (17-HSD) type 2 enzyme is the key enzyme in the inactivation of active estrogens and androgens. Experiments have well documented the important contribution of estrogen in breast cancer etiology. This study was designed to identify sequence variants in the *HSD17B2* gene and in order to verify their potential association with an elevated risk of breast cancer and/or modify the impact of *BRCA1/2* mutations. Then, the nucleotidic sequence of exons, intron-exon boundaries and the 5 and 3 non-coding regions of *HSD17B2* gene have been analysed among 63 breast cancer French-Canadian cases having strong family history of breast cancer. Twenty-six among them are carriers of a mutation in *BRCA1/2* gene. In the coding regions, three variants lead to a single amino acid substitution : Ala111Asp, Gly160Arg, Leu204Phe and three other are silent sequence variants : 318CT, 390GA, 936GA, all have been found in one individual. Moreover, we have also found six variants in the non-coding regions which are in strong linkage disequilibrium (D : 0.76 to 1) : -1287CG, -1047GC, -525TC, -400TC, -132CT, IVS6-57CG, with respective allele frequency of : 0.8%, 1.3%, 2.5%, 1.1%, 32.2%, 32.5%. There is also a CA repeat, designated -328(CA)<sub>4-9</sub>. By *in vitro* expression, the kinetic properties of the Gly160Arg mutant enzyme was similar to the wild-type enzyme, Leu204Phe mutant caused a partial loss of activity, whereas the mutant carrying Ala111Asp exhibit no detectable activity. In contrast to the Gly160Arg and Leu204Phe mutant proteins, no protein product could be detected by immunoblot analysis in cell extracts transfected with the Ala111Asp mutant vector. It is important to note that both Leu204Phe and Ala111Asp were not segregating with breast cancer, and the Leu204Phe mutation have been found in a *BRCA2* positive family, thus not supporting their potential association with breast cancer risk.

**Inducible Gene Expression Models for MEN2A and MEN 2B forms of RET.** *J.L.V. Shaw, S.D. Andrew, D.S. Richardson, S.M. Myers, J.L. Yome, L.M. Mulligan.* Pathology, Queen's University, Kingston, ON, Canada.

*RET* is a proto-oncogene, encoding a receptor tyrosine kinase with three C-terminal variants, RET 9, RET 43, and RET 51, each with its own unique carboxy-terminus, allowing for potentially distinct signaling properties. Germline mutations of *RET* are implicated in the cancer syndrome, Multiple Endocrine Neoplasia type 2 (MEN 2), which has two main subtypes, MEN 2A, and MEN 2B. Mutations in the extracellular domain of *RET*, usually involving cysteine residues, cause MEN 2A. A single point mutation in the tyrosine kinase domain of *RET*, causes MEN 2B, a more aggressive form of the disease. MEN 2A and MEN 2B mutations both result in altered *RET* activation, albeit by different mechanisms, which are not completely understood. In order to characterize these differences in MEN 2A and MEN 2B forms of all three *RET* isoforms, we have developed a two tiered, inducible model for *RET* expression. The first tier of control is transcription based, in which expression of the *RET* gene is controlled using a tetracycline inducible system. The second tier of the system allows for control of *RET* protein dimerization and subsequent phosphorylation and activation. We have created human embryonic kidney HEK derived 293 cells, which stably express these *RET* proteins upon induction. We have used this model to confirm the abilities of the induced proteins to bind several PTB domain containing, adaptor proteins, such as SHC and DOK1, which are known to bind to *RET*, by immunoprecipitation and GST pull down experiments. We are currently using these cell lines to evaluate differences in functions and interactions of the three *RET* isoforms, and the impact on these, of the MEN 2A and MEN 2B mutations. Our model represents a valuable tool to evaluate the underlying molecular events responsible for the genotype-phenotype correlations in the MEN 2A and MEN 2B forms of *RET*, in each of the *RET* isoforms.

**Increased genetic instability near rare HRAS1 minisatellite alleles in murine embryonic stem (ES) cells. G.**

Zhang, M. Isomura, A. Franck, G. Larson, L. Hsu, S. Ding, T. Krontiris. Dept Molecular Medicine, City Hope Beckman Res Inst, Duarte, CA.

Rare alleles of the HRAS1 minisatellite have been linked to a 2-fold increase in cancer risk. To examine potential pathogenic effects of rare alleles, we analyzed instability of human minisatellites in mouse ES cells. Targeting by homologous recombination, we placed three minisatellites the common progenitor, a1, and two rare alleles one repeat unit (a0.29) and two repeat units (a0.28) shorter than a1 downstream of the murine homologue, hras1. Heterozygotes were obtained by double targeting. Resulting clones were then subcloned under various conditions and genotyped to determine minisatellite allele status. No allele change was observed in a1(-neo)/wt, a0.29(-neo)/wt and a0.28(+neo)/wt ES cells. However, allele loss was observed in a1/a0.29(+neo) or a1/a0.28 (+neo) ES cells, which shown 16/96 a0.28 and 5/96 a0.29 alleles lost. In contrast, a1(+neo)/a0.29 ES cells didn't show any allele loss. In summary, hemizygous alleles were stable, regardless of the presence or absence of a neo gene in cis and regardless of mutation-inducing treatment such as irradiation or heat shock. When heterozygotes were examined, a very high rate of allele loss (7-17%) was observed. In every instance (21/21) the rare allele was lost. If the neo gene was instead on the progenitor a1 allele, the genotype remained stable. Selection with G418 also maintained the parental clone genotype. We concluded that the a0.28 and a0.29 alleles destabilized nearby regions if these regions had some nonhomologous sequences. Allele loss required the presence of a minisatellite on the homologue, implicating perhaps a recombinational intermediate. Possible implications for carcinogenesis will be discussed.

**Collagen type I gene mutations of alpha 1 chain (Col1A1) in Czech patients with Osteogenesis Imperfecta**

**Syndrome.** *O. Marikova*<sup>1, 2, 3</sup>, *I. Mazura*<sup>1, 3</sup>, *I. Marik*<sup>2</sup>, *D. Zemkova*<sup>2, 4</sup>, *M. Kuklik*<sup>2, 4</sup>, *S. Mazurova*<sup>1, 3</sup>, *D. Leznarova*<sup>5</sup>, *P. Capek*<sup>1, 3</sup>. 1) Anthropology and Human Genetics, Faculty of Science, Charles University, Prague 2, Czech Republic; 2) Ambulant Centre for Defects of Locomotor Apparatus, Prague, Czech Republic; 3) EuroMISE Cardio, Prague, Czech Republic; 4) 2nd Paed. Dept., University Hospital Motol, Charles University, Prague; 5) Institute of Forensic Medicine, Brno, Czech Republic.

The Ambulant Centre for Defects of Locomotor Apparatus is one of the places in Czech Republic where patients with various bone dysplasias are centralized. The authors would like to share more than 10 years experience with comprehensive treatment of severe cases of osteogenesis imperfecta (caused by mutations of genes coding chains of collagen type I on the chromosomes 7 (Col1A2) and 17 (Col1A1)). A group of 50 living patients with osteogenesis imperfecta syndrome (OI) is followed up in this centre. DNA bank (since 1998) contains 35 isolated DNA samples from patients with OI type IA, IB, III and IVB according to Sillence classification. Sequencing analysis was done in 23 samples for exons 17, 27, 30, 31 of COL1A1 gene and their side introns in our pilot molecular genetic study. Mutations were found in 14 patients of the studied group (10 females, 4 males). Most common were substitutions in exons, substitutions in introns No. 26 and 27 were on the second place. In one case was discovered insertion of 4 nucleotides in exon 30. We can't include mutations in other exons and introns. The molecular genetic results were confronted to clinical and anthropometric findings. We verify that more mutations in one genotype area in one patient are not in correlation with clinical severity of the disease. We didn't find any characteristics between compared patients with the same mutation. We share the idea of G. Cetta (Italy) that other genes, besides collagen genes, could interact the collagen production and become responsible for the severity of the outcome. DNA analysis of col1A1 gene exons is going on. Supported by grant of The Ministry of Education of Czech Republic EuroMISE - Cardio No. LN00B107.

**GLUTEN AND DAIRY RESTRICTED DIETS ALTER PLASMA AMINO ACIDS AND NEUROTRANSMITTER PRECURSORS IN AUTISM.** *G.L. Arnold, S.L. Hyman, L. Delucia, R.A. Mooney.*  
Department of Pediatrics and Pathology, University of Rochester School Medicine and Dentistry, Rochester, NY.

One popular alternative therapy for autism spectrum disorders (ASD) is the gluten-free/casein-free (GF/CF) diet, a regimen that eliminates milk and most grain products. It is hypothesized that natural opiate-like peptides found in these foods alter behavior in children predisposed to autism. Appropriately designed studies have not been published to confirm this hypothesis or demonstrate efficacy or long term nutritional safety of the diet. Prior pilot data have suggested that this diet might affect neurotransmitter precursors (of interest since altered tryptophan metabolism has been implicated in symptoms of ASD) or result in essential amino acid deficiencies. We studied 96 children aged 17 mos to 10 yrs diagnosed with ASD. Essential amino acids and the ratio of tryptophan to the sum of the other large neutral amino acids (tryp/LNAA - a measure of serotonin precursor availability) were reviewed. There were no significant differences between the control group of children (who had developmental delay without ASD) and the children with ASD on regular diets in essential amino acids. However, children on the GF/CF diet had significantly lower levels of isoleucine and leucine ( $p < 0.04$ ). There was also no significant difference in plasma alanine between the control group and the children with autism. The tryp/LNAA ratio was similar in controls and children with ASD on regular diets (0.11 and 0.12, respectively), but was significantly lower in the children with ASD on the GF/CF diet (0.096,  $p < 0.009$ ). The amino acid levels raise concern about the long term nutritional impact of the diet as practiced. The altered tryp/LNAA ratio merits further study in relation to how diet may alter neurochemistry and behavior or brain development in these children.

**A novel Frataxin mutation and unusual heterozygote expansion associated with a very late onset of FA. D.**  
*McDaniel, C. Woodley, L. Langford, S. Subromany.* Dept Surgery/Neurology, Univ Mississippi Medical Ctr, Jackson, MS.

Great majorities(98%) of patients with Friedreich's ataxia (FA) are homozygous for GAA repeat expansion in the first intron of the frataxin gene. The expansion is associated with disease presentation and a decrease in frataxin expression. Patients with smaller expansions have a later onset and lower rates of disease presentation. The remaining small population of FA patients are compound heterozygotes, with a GAA repeats on one allele and a point mutation on the other allele. Here we described a 51 year old male patient with clinical characteristics consistent with a very late onset of FA but with an unusual feature of heterozygote expansion of GAA repeats. The relationship of the expanded gene sequence to the severity of disease and age of onset presented here was not previously described. A Friedreich mutation analysis revealed normal allele with a GAA size of approximately 40 and the abnormal allele with an expansion size of approximately 800 repeats. CAG repeat sizes for SCA-1, -2, -3, -6, and 7 were normal. Sequence-based analysis of the frataxin gene found a single base substitution (A to G) on exon 4, causing a glutamine to arginine change at amino acid 148. Structurally, this change would result in the loss of a H-bond between the amide carbonyl of Gln148 in the beta-sheet spanning amino acids 142-148 and the backbone N of Gln153 in the beta-sheet from amino acid 153-158. The highly basic Arg would likely rotate away from Lys147, between Trp155 and into the protein surface. This would result in a looser structure and would add a positive charge in place of the neutral Gln. These changes may alter frataxin interactions with other proteins and diminish its role in Fe metabolism. In conclusion, it is not clear yet if substitution of arginine for glutamine would reduce the level of frataxin production. However, the confirmation of the FA gene mutation in this atypical case, broadens the clinical spectrum of FA and supports the use of molecular approaches for a definitive diagnosis and interpretation of unusual cases.

**Superficial Siderosis as a Cause for Progressive Late Onset Hearing.** *K.M. Dodson<sup>1</sup>, A. Sismanis<sup>1</sup>, W.E. Nance<sup>2</sup>.* 1) Otolaryngology, Virginia Commonwealth University, Richmond, VA; 2) Human Genetics, Virginia Commonwealth University, Richmond, VA.

Superficial siderosis is an important cause for progressive late onset hearing loss that is largely unrecognized by the genetics community. Hemosiderin deposition in the leptomeninges was first described as an incidental autopsy finding in 1908, but not until 1940 was its association with subarachnoid hemorrhage recognized, and subsequently confirmed by animal studies. The advent of T2 weighted MRI, which is sensitive to the paramagnetic properties of ferritin, has permitted the diagnosis in living patients by demonstration of the characteristic rim of hypointensity surrounding the brain stem and cerebellum. The 1st and 8th cranial nerves are especially vulnerable, because only short segments of these nerves are protected by a sheath of Schwann cells. Hearing loss is the most common clinical finding, along with anosmia, ataxia, pyramidal signs and dementia. Depending on the cause for the hemorrhage, focal neurologic signs including paralysis and seizures may be seen. Typically, a high frequency loss is noted initially, doubtless reflecting the tonotopic organization of axons within the 8th nerve. A remarkable feature of the syndrome is its long latent period. Our awareness of this condition was prompted by a 36 year old man with a 10 year history of progressive hearing loss who had a history of a craniotomy for a cerebellar cyst at four years of age with the subsequent development of radiologic findings of superficial siderosis. Isolated cases have been reported in patients with Marfan Syndrome and NF1, and multiple cases have been observed in families with cerebral cavernous malformation and hereditary transthyretin amyloidosis. Superficial siderosis should be considered as a pathogenic mechanism for any form of late onset progressive deafness especially those such as Alport, the Muckle Wells, Fehntner and Epstein Syndromes that can be plausibly linked to subarachnoid hemorrhage. Clearly, the deafness could be caused by genes that are not even expressed in the cochlea and would be missed by positional candidate screening strategies that rely exclusively on this property.

**Missense changes in USH1C in a Type 1 Usher patient?** *D.C. Blaydon<sup>1</sup>, B. Leroy<sup>2</sup>, S. Bhattacharya<sup>2</sup>, M. Bitner-Glindzicz<sup>1</sup>*. 1) Clinical and Molecular Genetics Unit, Institute of Child Health, London WC1N 1EH, UK; 2) Institute of Ophthalmology, London, EC1V 9EL, UK.

Usher syndrome type 1 is an autosomal recessive condition in which profound, congenital sensorineural deafness is found in association with vestibular hypofunction and childhood onset retinitis pigmentosa. The gene responsible for Usher type 1C, USH1C, encodes a protein containing three PDZ domains and mutations in this gene have also been shown to cause the nonsyndromic recessive deafness, DFNB18. There appears to be a genotype-phenotype correlation whereby frameshift, nonsense or splicing mutations in the 5' end of the gene give rise to Usher syndrome type 1C, while missense mutations affecting cochlea-specific exons and a splice site mutation leading to reduced levels of wild-type mRNA have been found in DFNB18 patients.

Here we report two sisters of Zulu Somalian descent diagnosed with Usher syndrome type 1 who have two novel missense changes in the USH1C gene. These changes are predicted to lead to conservative amino acid changes in the first and third PDZ domains of the USH1C protein, but they were not found in 100 normal controls. Haplotype analysis excluded linkage to CDH23 and PCDH15, the two genes in which mutations are the second most common cause of Usher type 1. However, linkage to both MYO7A, the gene responsible for 70% of Usher syndrome type 1 cases, and USH1C could not be excluded. Since the parents are dead, we explored a number of other strategies to try and determine whether the mutations in exon 5 and 18, 26.5kb apart in genomic DNA, were in cis or trans.

**WILDERVANCK SYNDROME . CLINICAL AND RADIOLOGICAL FINDING.** *M. Diaz-Garcia<sup>1</sup>, G. Garcia-Sanchez<sup>1</sup>, E. Martinez-Coria<sup>2</sup>, C.G. Santillan-Chapa<sup>3</sup>, C.F. Martinez-Cruz<sup>4</sup>, J. Moreno-Aguirre<sup>1</sup>.* 1) Depto. de Genetica.Instituto de la Comunicacion Humana/Centro Nacional de Rehabilitacion(CNR), Mexico, D.F; 2) Departamento de Tomografia.CNR; 3) Depto. de Rehabilitacion Pediatrica. CNR; 4) Servicio de Comunicacion Humana, Depto. de Seguimiento Pediatrico.Instituto Nacional de Perinatologia, Mexico, D.F.

The Wildervanck syndrome or Cervico-oculo-acoustic (COA) is a rare disorder characterized by a triad of features including congenital deafness (sensorineural, conductive, or mixed); fused cervical vertebrae (Klippel-Feil anomaly), and abducens palsy with retractio bulbi (Duane syndrome). Occasional abnormalities include mental retardation, occipital meningocele, pseudopapilledema, hydrocephalus, growth deficiency, cleft palate, ear abnormalities, cardiac defects, cervical ribs, absent kidney and cholelithiasis. This syndrome may be responsible for at least 1% of deafness among females, the deafness has been shown by radiologic studies to be due to a bony malformation of the inner ear and/or anomalies of the ossicular chain. The etiology is unknown. The disorder is limited, or almost completely limited, to females, raising the question of sex-linked dominance with lethality in the hemizygous male. An environmental etiology, due to a vascular disruption sequence during embryonic development, has been noted in Klippel-Feil, as in Moebius and Poland sequences. A combination of defects (Klippel-Feil and Moebius) could induce the more complex phenotype observed in COA syndrome. We present a 7-year-old male child with sporadic COA syndrome with right hemifacial microsomia, right microtia, low occipital hairline, limitation of ocular movement, abduction with retraction in both eyes (Duane type I), nystagmus, uvula bifida, submucous cleft palate, short neck, mirror movement of the upper extremities. Audiometry showed bilateral hearing impairment sensorineural profound. Ear CT scan, revealed a Mondini dysplasia. Radiography of the entire spine revealed a congenital fusion of C2 and C3 vertebrae, thoracic hemivertebrae, scoliosis, and spina bifida occulta (Klippel-Feil type III). MRI of cervical spine showed continuity of the vertebral body of C2 and C3.

**Palmoplantar hyperkeratosis with short stature, facial dysmorphism, and hypodontia: a case report.** *F. Castillo-Lorca<sup>1</sup>, G. Garcia-Sanchez<sup>1</sup>, M. Diaz-Garcia<sup>1</sup>, S.G. Juarez-Garcia<sup>1</sup>, C.F. Martinez-Cruz<sup>2</sup>, A.L. Torres-Rodriguez<sup>1</sup>.* 1) Departamento de Genetica, Instituto de la Comunicacion Humana, Centro Nacional de Rehabilitacion. Mexico, D.F; 2) Servicio de Comunicacion Humana, Departamento de Seguimiento Pediatrico, Instituto Nacional de Perinatologia. Mexico, D.F.

Seow in 1989 described 18 members of a family with a syndrome comprising palmoplantar hyperkeratosis, proportionate short stature, facial dysmorphism, clinodactyly, epilepsy, deafness, and hypodontia. This syndrome is inherited in an autosomal dominant manner with a high degree of penetrance but variable expressivity. This syndrome differs from the autosomal recessive types of palmoplantar hyperkeratosis such as Papillon-Lefevre syndrome which shows premature loss of both dentitions. It is also distinct from Unna-Thost syndrome in that it presents short stature, facial dysmorphism, and hypodontia. Seow suggest that these features found in the family that he studied may be a new syndrome. Case report: 23 years old female with moderate diffuse hyperkeratosis of the palms and soles, proportionate short stature, frontal bossing, ocular hypertelorism, clinodactyly of the fifth fingers, sensorineural bilateral hearing loss, and hypodontia. She has a family history of epilepsy and deafness. However she has not family history of the others features found by Seow.

**KEARNS-SAYRE SYNDROME.** *G. Garcia-Sanchez<sup>1</sup>, L.A. Ruano-Calderon<sup>2</sup>, C.F. Martinez-Cruz<sup>3</sup>, L. Hernandez-Gomez<sup>4</sup>, M. Diaz-Garcia<sup>1</sup>, A.L. Torres-Rodriguez<sup>1</sup>.* 1) Departamento de Genetica, Instituto de la Comunicacion-Centro Nacional de Rehabilitacion. Mexico, D.F; 2) Clinica de Enfermedades Neuromusculares.Instituto Nacional de Neurologia y Neurocirugia. Mexico, D.F; 3) Servicio de Comunicacion Humana, Departamento. de Seguimiento Pediatrico.Instituto Nacional de Perinatologia, Mexico, D.F; 4) Departamento de Audiologia.Instituto de la Comunicacion Humana/Centro Nacional de Rehabilitacion, Mexico, D.F.

Kearns-Sayre syndrome (KSS) is a mitochondrial rare disorder characterized by a triad of features including (1) onset in persons younger than 20 years; (2) chronic, progressive, external ophthalmoplegia; and (3) pigmentary degeneration of the retina. In addition, KSS may include cardiac conduction defects, cerebellar ataxia, and raised cerebrospinal fluid (CSF) Additional features associated with KSS may include myopathy, dystonia, endocrine abnormalities (eg, diabetes, growth retardation/short stature, hypoparathyroidism), bilateral sensorineural deafness, dementia, cataracts, and proximal renal tubular acidosis. Thus, KSS may affect many organ systems. KSS occurs secondary to deletions in mitochondrial DNA (mtDNA) that cause a particular phenotype. The gene in which deletions occur is identified as Online Mendelian Inheritance in Man number 530000. We describe a 27-year-old woman . She onset at 11-years-old with right ocular ptosis followed by right progressive external ophthalmoplegia. Muscular weakness. Onset at 26 years old. It has improved with Carnitina and Coenzina Q10 (CoQ10) administration. Progressive hearing loss onset at 24-years-old. At 27-year-old the audiogram shows sensorineural bilateral severe hearing loss and the ABR show bilateral no response at 100dB. Secondary amenorhea onset at 23 years old.It responding to hormonal administration. ECG at 26 year-old was referred as normal. Skeletal muscle biopsy shows ragged-red fibers.Squeletal muscular molecular test reported deletion in mitochondrial DNA (mtDNA).Pedigree. Her sister began at 19-years-old (2 months ago) with muscular weakness.

**WAARDENBURG SYNDROME. AUDIOMETRIC MANIFESTATIONS.** *L. Acosta-Ramos<sup>1</sup>, G. Garcia-Sanchez<sup>1</sup>, M. Diaz-Garcia<sup>1</sup>, C.F. Martinez-Cruz<sup>2</sup>, A.J. Moreno-Aguirre<sup>1</sup>, L. Hernandez-Gomez<sup>3</sup>.* 1) Departamento de Genetica.Instituto de la Comunicacion Humana/Centro Nacional de Rehabilitacion, Mexico, D.F; 2) Servicio de Comunicacin Humana, Departamento. de Seguimiento Pediatrico.Instituto Nacional de Perinatologia, Mexico, D.F; 3) Departamento de Audiologia.Instituto de la Comunicacion Humana/Centro Nacional de Rehabilitacion, Mexico, D.F.

Waardenburg syndrome (WS) is an inherited autosomal disorder. It was first described in 1951 as a new genetic entity characterized by: congenital sensorineural hearing loss, and pigmentary disturbances of the skin, hair and eyes. WS is classified as one of two types, according to the presence (type I) or absence (type II) of dystopia canthorum. La penetrance of the congenital hearing impairment (HI), the most significant clinical finding in patients with this syndrome, has been reported to 35 to 70%; in type I and 55 to 85%; in type II. When it occurs, the HI can be either complete or partial and bilateral or unilateral. Unilateral HL has been reported to be 4 to 13%;. Symmetrical HI 25%; of type I and 50%; of type II. A significant number of patients with WS have U-shaped audiograms. WS is inherited in dominant fashion. World-wide, approximately 1 in 42,000 infants is affected by WS. We studied 40 patients who had WS with HI, 14 male and 26 female. All of them were bigger than 3 years of age (mean 16 years). The patients were evaluated by conventional audiometric methods to determine the penetrance and the degree, type, symmetric, of HI. Ten (25%) of the patients had the type I of the syndrome and 30 (75%;) had type II. We found sensorineural profound HI in 90%; of type I and 83.3%; of type II. The symmetry was present in 60%; both type I and type II. The progression was only detected in one patient type II. Slowly descending configuration in 67.5%; for both types. Progression was only present in type II . Regarding the sex predominance the type I was only observed in the women. No significant statistical difference was found between patients type I and type II. We do not find significant statistical differences  $\chi^2=P>0.05$  in the audiometric manifestations between type I and type II of WS.

**JOHNSON McMILLIN SYNDROME: REPORT OF A NEW CASE.** *S.G. Juarez-Garcia<sup>1</sup>, G. Garcia-Sanchez<sup>1</sup>, M. Diaz-Garcia<sup>1</sup>, C.F. Martinez-Cruz<sup>2</sup>, L. Acosta-Ramos<sup>1</sup>.* 1) Departamento de Genetica, Instituto de la Comunicacion Humana/Centro Nacional de Rehabilitacion. Mexico, D.F; 2) Departamento de Seguimiento Pediatrico, Servicio de Comunicacion Humana.Instituto Nacional de Perinatologia, Mexico, D.F.

Alternative titles; symbols: Johnson-McMillin syndrome. Alopecia-Anosmia-Deafness-Hypogonadism syndrome. AADH syndrome. In 1983, Johnson described a 'new' autosomal dominant neuroectodermal syndrome in which anosmia and hypogonadotropic hypogonadism were combined with conductive deafness, alopecia, and other anomalies. In 3 generations, 16 persons were affected. Deafness was associated with protruding ears, microtia, and/or atresia of the external auditory canal. Variable features included congenital heart defect, cleft palate, mild facial asymmetry, facial nerve palsy, mental retardation, multiple truncal cafe-au-lait spots, hypohidrosis, tendency to caries and growth retardation. Inherited in an autosomal dominant pattern and with variable expressivity. We present a new case of Johnson McMillin Syndrome in a 2-year-old child, Mexican, gestational product III, with widespread alopecia, asymmetric ears: left microtia-atresia aural and right prominent ear, facial asymmetry, and red stains, his mother thinks that the child has anosmia or hiposmia, mild dental caries. ABR performed at 18 months of age reported bilateral hearing loss.

**Okhiro Syndrome: clinical features ; two cases in a Mexican Family.** *A.J. Moreno-Aguirre<sup>1</sup>, G. Garcia -Sanchez<sup>1</sup>, M. Diaz-Garcia<sup>1</sup>, L. Acosta-Ramos<sup>1</sup>, F. Castillo-Lorca<sup>1</sup>, C.F. Martinez-Cruz<sup>2</sup>.* 1) Departamento de Genetica.Instituto de la Comunicacion Humana/Centro Nacional de Rehabilitacion, Mexico, D.F; 2) Servicio de Comunicacin Humana, Departamento. de Seguimiento Pediatrico.Instituto Nacional de Perinatologia, Mexico, D.F.

Okhiro syndrome is an autosomal dominant inherited disease. The main features consists of Duane anomaly, radial ray defects and deafness. Deafness is congenital and usually sensorineural , polydactyly , hemifacial microsomia with skin tags, cardiac defects, and Hirschsprung disease have also reported.. Less frecuently reported anomalies are cervical vertebral anomalies, spinal and other skeletal abnormalities, palatal clefts and renal anomalies. Case A: The female proband 4 years old is the first child of young parents. She was an under diagnosed cause of deafness and squint.She presented as a neonate with bilateral hypoplasia external ear, with absent both thumbs, hypoplasia right radial and thenar, ventricular septum defect and Hirshchsprung disease. She has presented chronic middle otitis, courses with asymetrical hearing impairment (mild left and severale right conductive hearing loss) and right Duanes anomaly type I.Case B. Female 27 years old, probands mother . She was opered of bilateral syndactyly and courses with Bilateral Duanes anomaly.Mother and daughter, both have 46,XX, karyotype and molecular biology test in process.Okhiro syndrome must be differentiated with acro-renal-ocular syndrome, Fanconi syndrome, Holt-Oram syndrome, Wildervanck syndrome, 22q partial duplication and 22q11.2 deletion syndrome.

**Correlations Between Otovestibular Findings and Family History in Mexican Otosclerotic Patients. L.**

*Hernandez-Gomez<sup>1</sup>, S.G. Juarez-Garcia<sup>2</sup>, G. Garcia-Sanchez<sup>2</sup>, C.F. Martinez-Cruz<sup>3</sup>, M. Diaz-Garcia<sup>1</sup>, D.O. Gomez-Torres<sup>4</sup>.* 1) Departamento de Audiologia.Instituto de la Comunicacion Humana/Centro Nacional de Rehabilitacion, Mexico, D.F; 2) Departamento de Genetica.Instituto de la Comunicacion Humana/Centro Nacional de Rehabilitacion, Mexico, D.F; 3) Servicio de Comunicacin Humana, Depto. de Seguimiento Pediatrico.Instituto Nacional de Perinatologia, Mexico, D.F; 4) Departamento de Investigacion.Instituto de la Comunicacion Humana/Centro Nacional de Rehabilitacion, Mexico, D.F.

Background: Otosclerosis due to abnormal bone homeostasis of the otic capsule is a frequent cause of hearing loss in adults. Usually , the hearing loss is conductive, resulting from fixation of the stapedial footplate. And additional type of sensorineural hearing loss may be caused by otosclerotic damage to the cochlea. The etiology of the disease is unknown , and both environmental and genetic factors have been implicated. Objective: The goals of this study were to assess the prevalence of sporadic and familial forms of otosclerosis in a population of otosclerotic patients and to compare the findings between groups.Study Design and Setting: This retrospective/prospective study was conducted in a single institution.Patients: This study included 32 patients with diagnosis for otosclerosis.Main Outcome Measures: Clinical data, including pure tone audiograms ,tympanogram vestibular tests, computed tomography, were available in all the patients with otesclerosis. A letter was mailed to 100 patients to invite them to come to our Genetics Department for the realization of the pedigree. Results: A family history of otosclerosis was found in 21.87% of the patients. The otovestibular findings no differed between patients with a sporadic form and those patients with a familial form of otosclerosis (  $X^2=p > 0.05$ ).Conclusion:The variable expresivity in the OTSC could be due to the time of evolution and /or genetic heterogeneity.

**Fraser's "Northamptonshire Syndrome" - a distinct entity which mimics Pendred syndrome.** *W. Reardon<sup>1</sup>, D. Stephens<sup>2</sup>*. 1) Dept Clin Gen, Ctr Medical Gen, Our Lady's Hosp Sick Children, Dublin, Ireland; 2) Welsh Hearing Institute, University Hospital of Wales, Heath Park, Cardiff CF4 4XN, Wales.

The association of deafness and goitre in any patient rightly prompts consideration of Pendred syndrome as the likely diagnosis. There are occasional exceptions among which is the little known condition first alluded to by Fraser in his PhD thesis as the "Northamptonshire family." Fraser presented details of a six generation family, several individuals manifesting deafness. Reanalysis of the pedigree presented by Fraser shows several differences from the classical Pendred situation - 1) goitre and deafness occurred both separately and together, 2) affected individuals are seen in successive generations, 3) deafness was not congenital, 4) audiogram characteristics show a uniform loss across all frequencies, contrasting with the mainly high frequency loss seen in most cases of Pendred syndrome, 5) perchlorate test was normal in the 3 cases tested. In the course of identifying patients with Pendred syndrome we encountered 2 further families who present features consistent with the description of Fraser's "Northamptonshire Family." These observations have led us to conclude that there is a distinct clinical entity, largely unrecognised, which masquerades as Pendred syndrome. Initial molecular studies confirm neither evidence of mutation at the PDS locus nor linkage to that region of chromosome 7.

**ALOPECIA AREATA AND TURNER SYNDROME: AN UNCOMMON ASSOCIATION.** *H. Turki<sup>1</sup>, N.B. Abdelmoula<sup>2</sup>, M.F. Portnoi<sup>3</sup>, A. Saad<sup>5</sup>, A. Amouri<sup>4</sup>, T. Rebai<sup>1</sup>.* 1) service de dermatologie, CHU H. Chaker, Sfax, Tunisia; 2) laboratoire d'histologie, facult de mdecine de sfax, Tunisia; 3) laboratoire de cytogntique, hopital saint-antoine paris, France; 4) laboratoire de cytogntique, institut pasteur de tunis, Tunisia; 5) laboratoire de cytogntique, CHU f. Hached sousse, Tunisia.

Alopecia areata (AA) is a common form of non-scarring alopecia that appears equally in males and females of any age, although children and adolescents are more commonly affected. The vast majority of patients with AA are in excellent health and have no associated clinical conditions but a number of diseases have been reported showing increased prevalence in conjunction with AA for a minority of people such as Down's syndrome. We describe a 10-year-old female who was referred for a cytogenetic analysis because she developed an alopecia universalis. She had been found to have a mosaic 45,X/46,X,+r. Molecular cytogenetic analysis using sex chromosome probes permitted to identify the very small ring as a ring X chromosome which was detected in 90% of cells and appeared to be formed almost totally of alphoid sequences with breakpoints in the juxtacentromeric region. Using XIST probe at Xq13.2 the r(X) does not include the XIST locus and therefore, may not be subject to X-inactivation. The simultaneous occurrence of alopecia areata and Turner's syndrome has been reported by 4 authors (see references). As Turner's syndrome are frequently associated with autoimmune diseases or with serological markers for autoimmune diseases, an autoimmune origin has been suggested for alopecia areata. But, this uncommon association may indicate some genetic relationship between the two entities. Hypothesis of "skin mosaicism" can be also suggested to explain the association of the two entities in our patient.

**Prenatal diagnosis of Smith-Lemli-Opitz syndrome by triple screen of maternal serum.** *B.S. Reddy<sup>1</sup>, S.S. Reddy<sup>2</sup>.*

1) Department of Genetics, St. Joseph Medical Clinic, Detroit, MI; 2) Department of Internal Medicine, St. Joseph Medical Clinic, Detroit, MI.

Smith-Lemli-Opitz (SLO) is an autosomal recessive disorder. It has been known a defect in cholesterol biosynthesis is probable cause of this syndrome. VT was a 36 year old G3P2, referred at 16.2 weeks gestation for possible risk of having a fetus with Down's syndrome. The triple screen revealed that MSAFP was 0.41 MOM; unconjugated estriol 0.40 MOM and HCG 0.32. The patient was referred for amniocentesis. Karyotype revealed 46,XY. Biochemical analysis of amniotic fluid revealed that 7-dehydrocholesterol was greatly elevated when compared to control. Suspicion of early IUGR led the perinatologist to suggest elective termination of pregnancy. VT consulted for genetic counselling and she was explained about the biochemical and sonographic evidence taht was suggestive of SLO. The patient elected for termination of prgnancy. Autopsy revealed dolichocephaly, ambiguous genitalia with micropenis, hypospadias. Partial syndactyly the third and fourth toes bilaterally. Internal examination revealed VSD, ASD and bilateral unilobular lungs. This report demonstrates the role and importance of triple screen of maternal serum in the detection of fetus at risk for SLO.

**Characterizing genetic disorders: NF1 as a model for distinguishing between features, consequences and complications.** *E. Pivnick*<sup>1</sup>, *V. Riccardi*<sup>2</sup>. 1) Dept Ped, Div Clinical Gen, Univ Tennessee, Memphis, TN; 2) The Neurofibromatosis Institute.

In depicting a disorder, all too often, the specification, feature, is used without qualification, often subsuming primary, secondary and tertiary elements as simply features. We report here a system that distinguishes features as primary elements, consequences as secondary elements, and complications as tertiary elements. We have reviewed the clinical and radiological data from over 1,200 patients in the combined Baylor NF Program and The Neurofibromatosis Institute databases to identify anatomic and functional characteristics of the disorder, NF1. For example, vertebral dysplasia is a feature of NF1, while dystrophic scoliosis is a consequence of the vertebral dysplasia and spinal cord compression is a complication of the scoliosis. Distinguishing between these three levels is critical for understanding pathogenesis and for devising treatment strategies. For example, is the treatment directed at the feature, the consequence or a complication? For each of the many anatomic and functional elements of NF1 we will provide a table showing assignments to the feature, consequence and complication levels of involvement. This same line of reasoning obviously relates to a wide variety of genetic disorders and its delineation for NF1 therefore has wide application.

**INHERITANCE AND OSSEOUS DAMAGE BETWEEN OSTEOGENESIS IMPERFECTA TYPE III AND OSTEOPETROSIS IN TWO MEXICAN FAMILIES.** *J.M.R. Aparicio<sup>1,5</sup>, C.O. Gil<sup>2,5</sup>, M.P. Barrientos<sup>3</sup>, W.B. San Martin<sup>2</sup>, M.V.C. Mendez<sup>2</sup>, E.M. Landini<sup>4</sup>, J.A.R. Cao<sup>4</sup>, M.A.J. Enciso<sup>5</sup>, E.M. Huitzil<sup>5</sup>, C.F. Salinas<sup>6</sup>.* 1) Genetics; 2) Estomatology; 3) Endocrinology; 4) Ortopedics, Hospital para el Nino Poblano, Mexico; 5) Estomatology, Benemerita Universidad de Puebla, Mexico; 6) Oral genetics, medical University South Carolina, Charleston, usa.

**INTRODUCTION.** Are osseous dysplasia with dental aberrations due to an alteration of the conjuntive tissue. **OSTEOPETROSIS:** It is a rare genetic disease with both inheritance patterns, dominant ( 1:20 000) and recessive ( 1:200 000), characterized mainly due to a deficient metabolic reabsorption into the bone and cartilage. In relation to sex predisposition is 1;1. In relation to this disease, a defect in the bone shape and a high fracture incidence has been observed. As clinical features short size, anemia, microcefaly, fontocraneal bone increase and exoftalmos has also been documented, slow dental eruption, malformation and a high caries incidence was observed. Infections in this patients may give rise to osteomelitis. **CLINICAL CASE.** It is a seven years old female patient with multiple bone fractures in both legs, short size, exoftalmos, frequent headaches. **OSTEOGENESIS IMPERFECTA:** It is also a rare genetic disease (1:40 000 nb) due to a bone alteration within the collagen tipe I metabolism. In relation to the disease bone fragility, blue escleras, hipoacusia, opalescents dental color and articular hyper flexibility, prognatism, slow dental eruption, pulpar obliteration has been described. Osteogenesis type III is associated to dentinogenesis imperfecta. **CLINICAL CASE.** It is an eight years old female patient diagnosed with OI III. caries. **DISCUSION.** It is nowadays possible to find a major variability type of aberrations or syndromes with craniofacial alterations in relation to genetics. It may be important then to consider it as one of the primary cause of estomatologic and ortpedic diseases. In this study bone dysplasia was considered the most important eiologic factors in both studied patients. However, although both osseous diseases in this study have multiple fractures, the bone damage was totally different if compared the fractures type.

**Risk factors in limb reduction defects.** *Y. Alembik, B. Dott, M.P. Roth, C. Stoll.* Dept Genetique Medicale, Hosp de Hautepierre, Strasbourg, France.

**Objectives :** The aim of this study was to describe the prevalence at birth of limb reduction defects (LRD) and to identify possible etiologic factors. **Material and Methods :** The LRD came from 293,923 consecutive births of known outcome registered in the registry of congenital malformations of Strasbourg for the period 1979 to 2000. Request information on the child, the pregnancy, the parents, and the family was obtained. For each case a control was studied. The LRD was localised and classified according to the EUROCAT guide for the description and classification of limb defect. **Results :** The prevalence of LRD was 9.9 per 10,000 (291 cases). Non isolated malformations were present in 52.8% of the cases, 19 fetuses with LRD had an abnormal karyotype and 35 had a recognisable syndrome, association or an unspecified malformation pattern. Antenatal ultrasound examination was able to detect 101 cases (34.7%). The proportion of males was 0.55. The most common malformations in the 51.2% of children who had at least one other anomaly than LRD were cardiac, digestive and renal anomalies. Weight, length, and head circumference (HC) at birth of infants with LRD were less than those of controls. The weight of placenta of infants with LRD was less than the weight of placenta of controls. Pregnancies with LRD were more often complicated by threatened abortion, oligohydramnios and polyhydramnios but there were no differences in parental characteristics. However mothers of children with LRD took more often medications during pregnancy than mothers of controls and parental consanguinity was higher in parents of infants with LRD than in controls. First degree relatives also had more non-limb malformations than did those of controls. **Conclusions :** Weight, length, HC and weight of placenta of infants with LRD were less than those of controls. Pregnancies of infants with LRD were more often complicated by threatened abortion, oligohydramnios and polyhydramnios. Mothers of children with LRD took more often medications during pregnancy than mothers of controls. Parental consanguinity was higher than in controls and first-degree relatives of cases had more non-limb malformations than controls.

Program Nr: 530 from 2003 ASHG Annual Meeting

**The Hemi-3 syndrome, another case report.** *W.M. Eyaid.* Pediatrics, king Fahad Hospital, Riyadh, central region, Saudi Arabia.

The hemi-3 syndrome is a developmental syndrome that consists of hemihypertrophy, hemihypereasthesia, hemiareflexia, and scoliosis. We report on a 16 years old girl who was born with meningomyelocele. Physical examination revealed left sided hemihypertrophy, loss of pain sensation, decreased deep tendon reflexes and scoliosis. she had chronic left foot ulcer because of repeated trauma. Also, she had chronic urinary tract infection and required extensive urological operations. Neurophysiological studies revealed abnormal sympathetic skin response on the left side, slow sensory and normal motor nerve conduction velocities. Also, she had increased sensory threshold for the left-sided extremities compared to the right. The hemi-3 syndrome should be looked for in patients presenting with hemi hypertrophy to avoid the complications resulting from loss of pain and temperature sensation as in our patient.

**Generalized osteopenia in Neurofibromatosis Type I patients points to an underlying disorder of skeletal homeostasis and mineralization.** *N. Brunetti-Pierri<sup>1</sup>, K. Phan<sup>1</sup>, S. Carter<sup>1,3</sup>, R.A. Lewis<sup>1,2</sup>, S. Plon<sup>1,4</sup>, K.J. Ellis<sup>5</sup>, S.E. O'Brian<sup>5</sup>, B. Lee<sup>1,3</sup>.* 1) Dept Genetics, Baylor College of Medicine, Houston, TX; 2) Dept Ophthalmology, Baylor College of Medicine, Houston, TX; 3) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX; 4) Dept Pediatrics, Baylor College of Medicine, Houston, TX; 5) Children's Nutrition Research Center, Baylor College of Medicine Houston, TX.

Neurofibromatosis type 1 (NF1) is the most common autosomal dominant human disorder. Skeletal complications include macrocephaly, scoliosis, pseudoarthrosis, and short stature. However, bone mass in NF1 children and adults has not been systematically investigated. In a prospective observational study of NF1 subjects, we measured bone mineral status by dual energy X-ray absorbiometry (DXA). The cohort included 27 males [age 3.1 to 39.1 yrs] and 46 females [age 2.8 to 58.9 yrs] seen in an NF clinic and unselected for skeletal problems. The mean lumbar bone mineral density z score and the whole body bone mineral content z score were both significantly reduced [-1.381.05 and -0.611.19 respectively] ( $p < .001$ ). In a subgroup of 16 osteoporotic and osteopenic subjects, we evaluated the mechanism of osteopenia/osteoporosis with intact parathyroid hormone (PTH), 25(OH)vitamin D, calcium, phosphate, alkaline phosphatase, and markers of bone turnover. All markers were within the normal range except PTH, which was significantly increased from age-matched controls (mean PTH 58.325.6 ng/l) in 9 subjects. In those subjects, after conventional therapy with calcium and vitamin D, PTH decreased into the normal range for age (30.422.6 ng/l). Our data imply that NF1 patients are at risk for previously unrecognized osteopenia and osteoporosis without evidence of dietary vitamin D deficiency. Metabolic parameters and the therapy response in a subgroup of severely affected subjects suggest the presence of secondary hyperparathyroidism, perhaps due to underlying osteomalacia. A generalized defect in osteoblast and/or osteoclast function may be present in NF1, and additional longitudinal studies will determine long-term health inferences of these findings in older NF1 individuals.

**A new syndrome with skeletal dysplasia and mental retardation in two sisters.** *D. Genevieve*<sup>1,2</sup>, *D. Heron*<sup>2</sup>, *M. Le Merrer*<sup>1</sup>, *A. Jacquette*<sup>2</sup>, *D. Sanlaville*<sup>1</sup>, *F. Pinton*<sup>3</sup>, *N. Villeneuve*<sup>3</sup>, *G. Khalifa*<sup>4</sup>, *A. Munnich*<sup>1</sup>, *V. Cormier-Daire*<sup>1</sup>. 1) Department de Genetique, Hopital Necker-Enfants Malades, Paris, France; 2) Departement de genetique, Hopital de la Pitie-Salpetriere, Paris, France; 3) Service de Neuropediatrie, Hopital Saint Vincent de Paul, Paris, France; 4) Service de Radiologie, Hopital Saint Vincent de Paul, Paris, France.

Spondyloepimetaphyseal dysplasias (SEMD) are an heterogeneous group of conditions comprising at least 14 entities defined by clinical and radiological features. Here, we report on a new form of SEMD with mental retardation (MR) in two Pakistani sisters born to first cousin parents. The clinical phenotype is characterized by MR, ataxia and facial dysmorphism including coarse face, low anterior and posterior hairline, brachycephaly, simple and flat ears, synophrys, bilateral epicanthal folds, broad nose, full lips with eversion of the lower lip, short neck, and hirsutism on back and legs. Skeletal findings include flat vertebral bodies with irregular vertebral plates, irregular and flared metaphyses with vertical striations, small and irregular epiphyses, small carpal bones and narrow iliac wings without lacy pelvis iliac crest. Mental retardation in association with SEMD is observed in lysosomal storage disorder and Dyggve-Melchior-Clausen (DMC) syndrome. Extensive blood and urine metabolic screening was normal. Moreover, molecular studies of the Dymeclin gene responsible for DMC are currently performed. This combination of features observed in two sisters does not correspond to any reported case of SEMD and represents therefore a new autosomal recessive form of SEMD with MR.

**Aspondylodysostosis : delineation of a probably recessive spinal dysostosis with absent ossification of all vertebral bodies.** *M. Gonzales<sup>1</sup>, A. Verloes<sup>2</sup>, N. Joyé<sup>3</sup>, J.L. Taillemite<sup>3</sup>, P. Maroteaux<sup>4</sup>.* 1) Fetal Pathology Unit, Hôpital Saint Antoine, Paris, France; 2) Clinical Genetic Unit and INSERM E9935, Hôpital Robert Debré, Paris; 3) Embryology & Cytogenetics dept, Hôpital Saint Antoine, Paris; 4) Genetic Department, Hôpital Necker-Enfants Malades, Paris.

We report here 4 patients from 3 families with a striking and easily recognizable spinal anomaly characterized by total absence of the vertebral bodies, for which we suggest the name aspondylodysostosis. The first two patients were sibs born to consanguineous Maghrebian parents. Pregnancy of patient 1, a girl, was terminated after prenatal diagnosis of MCA. She had short trunk, short and wide thorax, spina bifida, and nephroblastomatous kidneys. Her younger sib had the same physical appearance, large kidneys, but no NTD. X rays were identical in both: total absence of vertebral bodies and sacrum associated with anarchic position of the vertebral pedicles, reduced number of ribs, narrow pelvis, with an upward widening of the iliac wings and unusual tilt of the the ischiopubic rami explained by pelvic anteversion. Appendicular skeleton was normal, as was the shoulder girdle. Patient 3 and 4 come to the term and died soon afterwards of respiratory insufficiency. Both had a radiological pattern similar to the former cases. Moreover, patient 3 had a cleft palate and patient 4 hypoplastic nails. Macroscopic examination at necropsy of case 1 indicated that the spine was not missing but rather replaced by a dense structure. These 4 patients appear to show the same vertebral dysostosis. They share similarities with the "polytopic anomalies with agenesis of the lower vertebral column" recently reported by Bohring, but differs by the extend of the vertebral defect, which goes higher than the thoracolumbar junction, and may thus represent a new dysostosis with possible AR inheritance.

**Genetics and periodontal health study in a four Iranian consanguineous families with Papillon-Lefevre Syndrome, Mashhad, North East of Iran.** *K. Ghodsi<sup>1</sup>, S. Banihashem Rad<sup>2</sup>, M. Tamizy<sup>2</sup>, R. Akbarzadeh<sup>1</sup>, M. Gorji Nejad<sup>1</sup>, M. Radvar<sup>2</sup>, A. Lotf Azar<sup>3</sup>.* 1) Dept Medical Genetics, Mashhad Univ Medical Sch, Khorasan, Iran; 2) Department of Periodontology 2, Dental School; 3) Department of Periodontology3, Dental School, Shiraz University of Medical Sciences, Iran.

**Introduction:**Papillon-Lefevre Syndrome (PLS) is an autosomal recessive palmoplantar keratodermal disorder which is characterized by hyperkeratosis of the palms, soles and severe early onset periodontitis. The frequency of PLS is approximately 1 to 4 per million. Genetic basis for most PLS appears to be mutations affecting both alleles of the cathepsin C gene (CTSC), located on chromosome 11q14.**Materials and Methods:**All families' members were clinically examined. A diagnosis of PLS was made in subjects with severe early onset periodontitis and the clinical appearance of hyperkeratosis on the palmar and plantar surfaces. Pedigrees were drawn and peripheral blood samples were taken. Lymphocytes were cultured and cells were harvested. GTG bandings chromosomes were analyzed. **Results:**Five cases of PLS in four families with almost 200 individuals in three generation diagnosed who were between 4.5 and 12 years of age. Parents were consanguineous. The pathological findings involve severe inflammation and destruction of the gingival as well as hyperkeratosis of the skin from palmar, plantar, and knee sites. Periapical radiographs showing severe alveolar bone loss affecting erupting permanent teeth. Atrophic changes of the nails, and a radiographic deformity of the fingers. None of the members of this family showed evidence of other systemic disorders and no other abnormality was detected. Chromosomal analysis of peripheral blood was performed after high resolution banding showed an apparently normal. **Conclusion:**The most PLS patients in Iran are from Mashhad and Shiraz so joining projects to study clinic and genetics were planned. First cousin marriages may suggest recessive inheritance for PLS in these families. Although cytogenetics did show any chromosomal abnormalities, to study molecular genetics DNA from patients and families were extracted.

**Dominant distal arthrogryposis in a 2 generation family: clinical and genetic analysis.** *P. Bitoun*<sup>1</sup>, *E. Pipiras*<sup>2</sup>, *B. Benzacken*<sup>2</sup>, *J. Gaudelus*<sup>3</sup>. 1) Genet Medic, CHU Paris-Nord, Hosp Jean Verdier, Bondy, France; 2) Embryo-Cytogenetics and Reproductive Biology Dept; 3) Pediatrics Dept.

Arthrogryposis has been defined as limitation of joint motion of 2 or more joints and results from multiple causes from uterine mechanical constraint, oligohydramnios to iatrogenic, ligamentous, neurogenic atrophy or myopathic pathology. Dominant distal arthrogryposis has been reported in several families. We report a 2 generation family. Purpose: to describe and search for the genetic etiology of dominant distal arthrogryposis. Material and methods: Family with distal arthrogryposis was examined and photographs, radiographic studies and blood samples were taken for lymphoblastic cell lines and informed consent was granted for molecular genetic research. We present the case of a mother with congenital distal arthrogryposis with surgically corrected club feet, limited range of motion of hands (camptodactyly and ulnar deviation) and elbows, scoliosis, hypertelorism and short stature. She gave birth to 2 affected sons with normal intelligence who presented with oligohydramnios, club feet and club hands with clino and camptodactyly and hypertelorism. The 5 year old has had surgical correction for the club feet and the 1 year old is treated with strapping pending surgical correction. They both have hypertelorism but no scoliosis and are responding well to physical therapy. The more severe affection of the mother suggests dominant inheritance; there is no microstomia suggestive of Freeman-Sheldon syndrome. Results: We describe the clinical and radiologic phenotype of 3 affected members of a Dominant distal arthrogryposis family. Molecular analysis by Sung et al have recently identified genes encoding fast-twitch contractile proteins TNNI2 and TNNI3 involved with DA2B and TPM2 with DA1. Mutation analysis will be performed in this family to try to identify the genetic etiology.

**Spontaneous cerebrospinal fluid leaks: Part of the Marfan spectrum, or a new entity?** *O. Gordon*<sup>1</sup>, *J. Tourje*<sup>3</sup>, *W. Schievink*<sup>2</sup>. 1) GenRISK Program, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) Maxine Dunitz Neurosurgical Insitute, Cedars-Sinai Medical center, Los Angeles, CA; 3) Imaging Medical Group, Cedars-Sinai Medical center, Los Angeles, CA.

Spontaneous spinal cerebrospinal fluid (CSF) leaks are the most common cause of spontaneous intracranial hypotension and postural headaches. The exact cause of spontaneous spinal CSF leaks is unknown, but a structural dural weakness predisposes to the formation of fragile menigeal diverticula or dural rents that allow CSF to leak out. Previous case reports and a retrospective study have found evidence for a systemic connective tissue disorder in some patients. We prospectively evaluated a group of patients with CSF leaks for evidence of a generalized connective tissue disorder. **Methods:** 18 consecutive patients referred for surgical repair of a spontaneous CSF leak were evaluated via comprehensive physical examination, echocardiography, skin biopsy and CT myelogram. **Results:** The mean age of the 15 women and 3 men was 38 years (22 to 55). Three patients demonstrated a Marfanoid habitus (height >95%, U/L ratio <.8, and arachnodactyly), and mild to moderate hypermobility. Imaging revealed no vertebral column deformities, dural ectasia or pulmonary blebs. All had multiple menigeal diverticula. None had myopia or lens dislocation, and echocardiogram was normal. Two patients had normal stature but marked hypermobility of large and small joints and were found to have markedly attenuated dorsal muscular fascia at the time of surgical repair, precluding proper wound closure. MRI revealed dural holes or rents. Echocardiography revealed mitral valve prolapse in one patient. Five patients had isolated moderate to marked small joint hypermobility, and the remaining six had no associated findings. **Conclusions:** Dural ectasia is a common and highly sensitive diagnostic finding in Marfan syndrome, but spontaneous CSF leaks have not been described. Our study reveals a broad spectrum of connective tissue abnormalities associated with the development of spontaneous CSF leaks. Further research is warranted to investigate possible fibrillin or collagen abnormalities in this group of patients.

**Achondroplasia-hypocondroplasia complex in a Mexican patient.** *A. Gonzalez-del Angel<sup>1</sup>, E. Spector<sup>2</sup>, MA. Alcantara<sup>1</sup>, V. del Castillo<sup>1</sup>.* 1) Departamento de Investigacion en Genetica Humana, Instituto Nacional de Pediatria, Mexico City, Mexico; 2) Department of Pediatrics, University of Colorado Health Sciences Center, USA.

Achondroplasia (ACH) is the most common short-limb skeletal dysplasia with an autosomal dominant inheritance. Most of the cases result from a de novo mutation in the fibroblast growth factor receptor 3 gene (FGFR3) that causes a substitution of an arginine for a glycine at position 380 (G380R/G1138A). The most frequent clinical manifestations are rhizomelic limb shortness, enlarged head with frontal bossing, midface hypoplasia, and short, broad hands with a trident configuration. Hypocondroplasia (HCH) is considered an allelic disease of ACH with a less severe phenotype. The mutation observed in about 70% of HCH patients is a transversion at nucleotide 1620 (C1620A or C1620G). The phenotype of patients with the achondroplasia-hypocondroplasia complex (ACH-HCH complex) is not well defined, because there are only three patients reported in the literature with a confirmed diagnosis by molecular studies. We report a Mexican patient whose mother has ACH and the father HCH, neither had genetic counseling or molecular testing prior to when the patient was born. The index case was studied at the National Institute of Pediatrics when he was 11 months of age, he had a phenotype of ACH patients but he also had important respiratory difficulties and he required surgical management due to an abnormal size and shape of the foramen magnum. He has been hospitalized 11 times due to bronchopneumonias and in one occasion he had a cardiac arrest. He is now 4 years old and has a severe developmental psychomotor delay. The clinical manifestations and the evolution of the patient suggest that he could have the ACH-HCH complex, and was confirmed by molecular studies that showed he is a compound heterozygous (G1138A/C1620G). The clinical evolution of the patient indicates that the phenotype of the ACH-HCH is more severe with important neurological and respiratory complications than observed in ACH or HCH patients.

Program Nr: 538 from 2003 ASHG Annual Meeting

**Wolcott-Rallison syndrome in a Bedouin boy: a brief clinical report.** *M.J. MARAFIE, A.R. Mary, R.L. Alnaggar.*  
Obstetric&Gynecology, Kuwait Medical Genetics Center, Kuwait.

We present a 7-year old Bedouin boy with short stature due to epiphyseo-metaphyseal dysplasia, infancy onset insulin-dependant diabetes mellitus, osteoporosis, recurrent hepatitis, seizures and developmental delay. He had additional manifestations such as microcephaly, mental retardation, hypothyroidism and skin/teeth changes. His karyotype was 46,XY. The clinical and radiological findings are consistent with the autosomal recessive mode of inheritance. This syndrome might be underestimated in the population of Arab peninsula, possibly due to early death of diabetic infants and the late expression of the full clinical picture.

**Comparison of Height and Thoracic Circumference Measurements of 20 Spondylothoracic Dysplasia Patients with the General Population.** *J.M. Massanet<sup>1</sup>, S.E. Carlo<sup>1</sup>, N. Ramirez<sup>2</sup>, A.A. Reis<sup>1</sup>, H. Rivera<sup>1</sup>, J.R. Acevedo<sup>3</sup>, V. Franceschini<sup>1</sup>, A.S. Cornier<sup>1</sup>.* 1) Genetic Division, Ponce School of Medicine, Ponce, PR; 2) Department of Orthopedics, Mayaguez Medical Center, Mayaguez, PR; 3) Dept. of Nursing, Univ. of PR at Arecibo, Arecibo, PR.

Spondylothoracic dysplasia (STD; MIM#277300) is a genetic disorder with autosomal recessive inheritance. Findings include segmentation and formation defects throughout cervical, thoracic and lumbar spine such as hemivertebrae, block vertebrae, and unsegmented bars with fusion of all the ribs at the costo-vertebral junction (crab-like chest configuration). We have followed 20 patients, of which 11 were male and 9 were females, ranging from 1 day to 13 years old, in our genetics clinic. We measured the thoracic circumference and the height, later correlating the values with their percentiles for the respective ages. Both the average chest circumference and the average height were lower than the general population with values in the 30.85th and 2.33th percentiles respectively for the entire group. These values were statistically significant with a p value of 0.008 for chest circumference and 0.001 for the height. The average female measurements were the 33.89th percentile for the chest circumference Vs 28.36th percentile for males, and 3.11th percentile for height Vs 1.27th percentile for males. These results show that these patients are well below the average height for their age. Their chest circumference measurements were also below average. However, it falls within less than one standard deviation below the general populations mean, whereas the height percentile falls more than 2 standard deviations below the general populations mean. This leads us to conclude that the chest circumference is less affected than the height in this syndrome, and that clinically the patients appear to have a large chest circumference for their height.

**A novel missense mutation in the FGFR3 gene causing hypochondroplasia.** *S. Heuertz<sup>1</sup>, M. Le Merrer<sup>1</sup>, V. Cormier-Daire<sup>1</sup>, L. Legeai-Mallet<sup>1</sup>, E. Flori<sup>2</sup>, A. Munnich<sup>1</sup>, J. Bonaventure<sup>1</sup>.* 1) Medical Genetics, INSERM U 393, Paris, France; 2) CHU Strasbourg, Hopital Hautepierre, 67098 Strasbourg cedex.

Hypochondroplasia (HCH) belongs to a clinical spectrum of skeletal dysplasias comprising HCH, achondroplasia (ACH), Severe Achondroplasia with Developmental Delay and Acanthosis Nigricans (SADDAN) and thanatophoric dysplasia (TD). All these dysplasias are accounted for by recurrent missense mutations in the FGFR3 gene. While FGFR3 mutations are routinely identified in more than 98% of ACH and TD patients, this percentage is much lower in the case of HCH owing to the difficulty to accurately diagnose this condition. Screening a large series of HCH patients including 65 sporadic cases and 18 familial forms for FGFR3 mutations, we identified the most common N540K mutation in 47 patients (57%). One patient carried the K650Q mutation in the tyrosine kinase 2 (TK2) domain of the receptor and a novel V381E mutation in the transmembrane domain was identified in one case. Interestingly, this mutation is adjacent to the G380R achondroplasia mutation and equivalent to the V644E mutation that occurs in the transmembrane part of the tyrosine kinase receptor Neu, causing its constitutive activation into an oncogene. Clinically, the V381E substitution was associated with a relatively mild phenotype characterized by normal size (52 cm) at birth and an absence of macrocephaly. Later on, statural retardation was moderate (-1.5 SD). Radiographs at eight years showed mild flaring of the metaphyses and slightly reduced interpedicular distances between lumbar vertebrae whereas phalanxes had a normal size. Since the K650Q mutation was also associated with a moderate phenotype, we suggest that HCH mutations outside the TK1 domain could result in milder phenotypes than the N540K mutation. This work was supported by the European Skeletal Dysplasia Network (ESDN).

**Agenesis of the parietal bones: Cleidocranial dysplasia or unique skeletal dysplasia.** A. Hing<sup>1</sup>, K. Leppig<sup>2</sup>, M. Seto<sup>1</sup>, M. Cunningham<sup>1</sup>. 1) Division of Genetics and Development, Univ Washington, Seattle, WA; 2) Genetic Services, Group Health Cooperative, Seattle, WA.

Congenital defects in cranial ossification can be observed in metabolic bone disease, parietal foramina permanga, and skeletal dysplasias such as Cleidocranial dysplasia. Congenital absence of the parietal bones is a rare anomaly that can be seen in isolation or in association with multiple birth defects or generalized bone dysplasia. We present clinical, radiographic, and molecular findings in a male child born with agenesis of the parietal bones and progressive skeletal deformity. The proband was delivered by Cesarean section due to fetal distress at term to a healthy female. Pregnancy was complicated by in utero demise of a twin fetus at 8-10 weeks gestation. At birth he was noted to have absence of skull bone by palpation. Radiographs showed minimal frontal and occipital bone ossification, normal clavicles, and undermineralization of the pubic symphysis. Laboratory studies showed normal calcium and thyroid studies with transient low alkaline phosphatase. A high-resolution CT scan at two months of age showed complete absence of the parietal bones with abnormal occipital bone formation. CT images at 8 months of life showed symmetric islands of parietal bone formation, multiple small ossific island of occipital bone formation, and absent nasal bones. Repeat alkaline phosphatase levels were elevated. Clinical examination was significant for progressive hypertelorism and chest wall deformity with increased anterior-posterior dimension. Linear growth and neurologic development were normal. Chest radiograph showed poorly mineralized bones with broad ribs. Family history was notable for short stature and a late-closing anterior fontanelle in the father and other paternal relatives. We present serial radiographic evidence of a skeletal dysplasia and progressive symmetric membranous bone ossification in a child born with complete agenesis of the parietal bones. We compare radiograph findings in this child with those seen in individuals with Cleidocranial dysplasia and parietal foramina permanga, and discuss results of *Msx2*, *Alx4*, and *Cbfa1* mutational analysis.

**Clinical Genetic, Radiological, & Molecular Analysis of Congenital Scoliosis and Kyphosis.** *M.K. Maisenbacher*<sup>1</sup>, *J.-S. Han*<sup>1</sup>, *E.H. Zackai*<sup>1,3</sup>, *J.P. Dormans*<sup>2,4</sup>, *B. Erol*<sup>2</sup>, *M.R. Tracy*<sup>1,2</sup>, *P.D. Turnpenny*<sup>5</sup>, *K. Kusumi*<sup>1,2,3,4</sup>. 1) Div Hum Genet & Mol Biol; 2) Div Orthop Surg, Children's Hosp of Philadelphia, PA; 3) Dept Ped; 4) Dept Orthop Surg, Univ of Penn School of Med, Philadelphia, PA; 5) Royal Devon & Exeter Hosp, Exeter, UK.

Congenital vertebral malformations occur in the context of genetic syndromes such as VATER association and Goldenhar syndrome, as well as non-syndromic congenital scoliosis and kyphosis. The genetic etiology of congenital scoliosis is largely unknown. Recently, mutations in the notch ligand *DLL3* have been identified in the vertebral disorder, spondylocostal dysostosis, and studies in the mouse have identified that notch pathway disruptions lead to vertebral defects.

To identify diagnostic subgroups, we analyzed radiological, clinical genetic, and molecular data from 74 infants and children with congenital vertebral malformations, divided into 32 syndromic (including VATER, Goldenhar) and 42 nonsyndromic (congenital scoliosis and kyphosis) cases. Radiologically, cases were analyzed by the extent of segmental defect, into vertebral dysostosis type 1 (global), type 2 (multiple vertebrae affected in 1 region), and type 3 (single). In 8 syndromic cases, we identified 6 type 2 (75%) and 2 type 3 (25%) defects. In 29 non-syndromic cases, we identified 17 type 2 (59%) and 12 type 3 (41%) defects. Clinical genetic analysis of the 42 nonsyndromic congenital scoliosis patients identified a high rate of nonskeletal associations, including genitourinary (14%), cardiovascular (10%), and neurological (17%) systems.

Molecular sequence analysis of notch pathway genes was carried out for 18 syndromic patients, and sequence polymorphisms were identified: *DLL3* (8 cases with synonymous and 4 with amino acid changes), and *HES7* (8 cases with synonymous changes). Molecular analysis of 27 nonsyndromic patients identified polymorphisms: *DLL3* (13 cases with synonymous and 4 with amino acid changes), and *HES7* (5 cases with synonymous and 1 with amino acid changes). Comparison with the general population and identification of multiplex families is underway, to elucidate the significance of these findings.

**Bone dysplasias in spontaneous abortions: Thanatophoric Dysplasia (TD) with unusual anomalies.** *V. Moran<sup>1</sup>, I. Garcia Pelaez<sup>1, 2, 4</sup>, C. Ortiz<sup>1, 3</sup>, H. Perez<sup>1, 2</sup>, O. Aguirre<sup>2</sup>, L.R. Cornejo-Roldan<sup>2</sup>, D. Saavedra<sup>2</sup>, M. Arteaga<sup>1, 2, 4</sup>.* 1) School of Medicine, Univ Panamericana, Mexico City, Mexico; 2) Hospital Infantil de Mexico; 3) Hospital ABC; 4) Faculty of Medicine, National Autonomous University of Mexico.

**INTRODUCTION:** Bone dysplasias have wide phenotypic expression that complicates its diagnosis specially in embryos and fetuses as some times few cases have been reported and some may be lethal varieties not usually seen. We present the case of a fetus with a probable TD with unusual anomalies. **CASE DESCRIPTION:** The fetus was product of the 5th gestation of young, non-related parents. The mother suffered previously from diabetes mellitus and hypertension and had vaginal and urinary infections during the pregnancy; while pregnant, she took prescribed drugs for chronicle and acute illnesses. The 2 previous pregnancies ended in spontaneous abortions. Pregnancy weeks are unknown, the last menstrual period date coincided with the last abortion, the mother was seen in consultation 6 months after it. The case was a male, 14 wks of gestational age by measures, with macrocephaly, turri-dolicocephaly, left ablepharon, right eyelid coloboma, anteverted nostrils, mild syndactyly, hypoplastic pelvis and legs, a discrete outlined sacral tail and hypertrophic left parotide region. Karyotype analysis was not available. The radiology showed a turri-dolicocephaly skull, abnormal ribs and severely bowed femura. Necropsy showed a liquid collection in parotid region, hypoplastic aortic arch, thymus hypoplasia, ectopic left kidney and Meckel diverticulum. The femur histology showed an irregular growth plate, big transversal blood vessels, fibrous ring and clusters of osteoblast without bone matrix formation. **DISCUSSION.** The diagnosis considered was a bone dysplasia probably of a TD type I but this case shows other characteristics not usually seen. The bone histology is shared with other bone dysplasias, and there is also a lethal variety of achondroplasia with thymus hypoplasia and bowing of the femura, however our case has also other anomalies. We consider that the mother illnesses or the drugs treatment do not support a teratogenic effect.

**BLAU SYNDROME AND THE COMPLEXITIES OF PROPER DIAGNOSIS AND TREATMENT.** *S. Kleppe*<sup>1</sup>, *D. Metry*<sup>2</sup>, *C. Bacino*<sup>1</sup>. 1) Dept Genetics, Baylor Col Medicine, Houston, TX; 2) Dept dermatology, Baylor Col Medicine, Houston, TX.

Blau syndrome is an autosomal dominant condition characterized by multi-systemic non-caseating granulomatous inflammation. Clinically Blau syndrome is manifested by arthritis, uveitis and a maculo-erythematous skin rash. This disorder can be easily confused with sarcoidosis or juvenile reumathoid arthritis (JRA). Blau syndrome is caused by mutations in the NOD2/CARD15 gene (caspase recruitment domain containing protein 15) that has recently been mapped to 16q12. Blau syndrome is a rare disorder and to this date there are very few reported families with Blau syndrome. Because of that, very little is known about treatment approaches to this disease. We report a mother with her two sons affected with Blau syndrome that were mis-diagnosed and treated for JRA with steroids and methotrexate with no response to the treatment and multiple adverse reactions to the drugs used. On further evaluation, a skin biopsy done in one of the affected children showed non-caseating granulomatous lesions. The possible diagnosis of sarcoidosis was raised, but could not be proved due to unknown etiology and the absence of a specific diagnostic test. Given the clinical presentation Blau syndrome was suspected and mutation analysis of the NOD2/CARD15 gene was undertaken. Mutaton studies showed a previously reported Arg334Gln heterozygous missense mutation in the centrally located nucleotide-binding domain, where most of the Blau syndrome mutations where found in the past. In contrast with CARD15 alleles associated with Crohn disease that affect the leucine rich domain (LRR) that interacts with bacterial peptidoglycan (PGN) to form the inflammatory granulomas, alleles associated with Blau syndrome promote PGN-independent Nuclear factor Kappa B (NFkB) activation, a finding that explains the affected organs in each condition. Tumor Necrosing Factor (TNF)-alpha is a direct regulator of NFkB. The treatment with Etanercept, a TNF antagonist, showed immediate relief of multiple symptoms in the affected individuals. This case illustrates the importance about knowing the gene defect and its function to tailor an appropriate treatment.

**Multiple exostoses, mental retardation, dysmorphic facies in a girl with no detectable mutation in**

**EXT1,EXT2,and TRPS1.** A. Shanske<sup>1</sup>, H.-J. Ludecke<sup>2</sup>. 1) Children's Hospital at Montefiore, Albert Einstein College of Medicine, Bronx, NY; 2) Institut für Humangenetik, Universitätsklinikum, Essen,Germany.

Multiple exostoses is a genetically heterogeneous disorder. *EXT1* on 8q24.1 and *EXT2* on 11p11-12, have been identified and a third locus, *EXT3*, has been assigned to 19p. Multiple exostoses are also observed in two contiguous disorders: the Langer-Giedion syndrome or TRPS II caused by the simultaneous deletion of the *EXT1* and *TRPS1* genes and the proximal 11p deletion syndrome (P11pDS) associated with the deletion of *EXT2* and *ALX4*. Here we report on a 10 and 2/12 year old girl with multiple exostoses, mild mental retardation and dysmorphic features. She was the 2.7 kg product of a term uneventful pregnancy. Her height, weight and head circumference were all 2-4 SD's beneath the mean. She had brachycephaly and a low anterior hair-line, thick brows with a synophrys and a bulbous nasal tip. The eyes were deep-set and the ears were large. She had a slight levoscoliosis, winging of the right scapula and hyperextensibility of all small and large joints. She had syndactyly of the 3rd and 4th fingers of both hands and the 2nd and 3rd toes bilaterally and brachydactyly of both 5th metacarpals. The bone age was greatly delayed between 6 and 7 SD's below the mean and cone-shaped epiphyses were present at multiple sites, predominantly in the middle phalanges. She had multiple exostoses of both humeri, left femoral neck and the left scapula. High resolution karyotyping revealed normal chromosomes and in situ hybridization showed ish 8q24.1(*EXT1*x2,*TRPS1*x2), 11p11.2(*EXT2*x2), and the patient was heterozygous for three *TRPS1* intragenic polymorphisms. These results exclude the deletions typically found in TRPS II or P11pDS. We could not detect any mutation by sequencing the entire *TRPS1* protein coding region. Because the patient's appearance resembles neither the TRPS nor the P11pDS closely, we must consider several alternative etiologies: 1) one mutation in one of the EXT genes and one mutation in the promoter or non-coding region of *TRPS1*, or in an unknown gene that results in a TRPS-like phenotype. 2) an undetectable paracentric inversion of 8q24 effecting *EXT1* and *TRPS1*. 3) a contiguous gene syndrome involving *EXT3* on 19p.

**Chondrodysplasia punctata and maternal autoimmune disease: a new case and review of the literature.** *N. Shur, R. Marion, C. Vega-Rich, A. Shanske.* Center for Congenital Disorders, Children's Hospital at Montefiore, Albert Einstein College of Medicine, Bronx, New York.

Chondrodysplasia punctata (CDP) is a rare but well-known skeletal abnormality characterized by irregular punctate calcifications. It is seen in a heterogeneous group of disorders including disorders of cholesterol or plasmalogen metabolism. It is also seen following maternal exposure to warfarin, dilantin, phenytoin, and alcohol as well as in rubella embryopathy and deficiency of vitamin K. There have been three previous reports of a direct association with a maternal autoimmune disease. We report a new case and review the literature. A male infant was the second child born of healthy unrelated parents. There was a history of 3 spontaneous abortions at 4 months gestation and he was delivered by SVD after a 36 week gestation with a birthweight of 2176 gm and length of 42 cm and HC of 33 cm. He had midfacial hypoplasia, an upward obliquity of the palpebral fissures, and bilateral epicanthal folds. There were no skin lesions. He had rhizomelic shortening of the arms and legs and brachydactyly of the fingers. A skeletal survey demonstrated stippling of all of the vertebrae, carpal bones, phalanges, knees, hips and tarsal bones. Biochemical studies revealed no abnormalities associated with CDP with normal plasmalogen, 8(9)cholestenol, and very long chain fatty acid levels. When last seen at 22 months, he was in good health and demonstrating mild developmental delays. However, his mother was noted to have a butterfly rash and dermatitis of the dorsum of both hands. Serologic investigation and dermatopathology were consistent with discoid lupus erythematosus. The 3 previous reports described either stillborns or a newborn with features of neonatal lupus all born to mothers with SLE. Further investigation of the mother may give further insights into this unusual pathogenetic mechanism of CDP.

**Differences of Anthropomorphic Measurements in the Survival Probability of 20 Patients with Spondylothoracic Dysplasia.** *A.A. Reis<sup>1</sup>, J.M. Massanet<sup>1</sup>, S.E. Carlo<sup>1</sup>, N. Ramirez<sup>2</sup>, V. Franceschini<sup>1</sup>, H. Rivera<sup>1</sup>, J. Acevedo<sup>1</sup>, A.S. Cornier<sup>1</sup>.* 1) Genetic Division, Ponce School of Medicine, Ponce, PR; 2) Department of Orthopedics, Mayaguez Medical Center, Mayaguez, PR.

Spondylothoracic dysplasia (STD; MIM#277300) is a pleiotropic genetic disorder with autosomal recessive inheritance. Findings include segmentation defects throughout cervical, thoracic and lumbar spine such as hemivertebrae, block vertebrae, and unsegmented bars with fusion of all the ribs at the costo-vertebral junction (crab-like chest configuration). It was generally regarded as a lethal condition in the neonatal period. However, we have followed 20 patients from birth, of which 8 died within the first 6 months of life. This group consisted of 5 males and 3 females, with an average age of death of 58 days. The group that survived consisted of 6 males and 6 females, ranging from 6 months to 13 years old, and with an average age of 5 years old. We measured the thoracic circumference at the nipple level and the height, later correlating the values with their percentiles for the respective ages, which is available in the literature for children up to 14 years old. Both the average chest circumference and the average height were lower than the general population with values in the 30.85th and 2.33th percentiles respectively. These values were statistically significant with a p value of 0.008 for chest circumference and 0.001 for the height. The average measurements for the group that did not survive were the 35.38th percentile for the chest circumference Vs 28.83th percentile for survivors, and 1.13th percentile for height Vs 2.75th percentile for survivors. These differences were not statistically significant (p value of 0.539 for chest circumference and 0.209 for the height), probably due to the small size of our sample. This data demonstrate that the non-survivor group was more disproportionate with a shorter height and a larger chest circumference. The shorter height does not allow for diaphragmatic descent, which may be compensated by a increase in the thoracic circumference. However, the fusion of the ribs may not permit a sufficient chest expansion to be compatible with survival.

**Sequential Pulmonary Function Tests in 5 Spondylothoracic Dysplasia Patients.** *N. Ramirez<sup>1</sup>, J.R. Acevedo<sup>3</sup>, S.E. Carlo<sup>2</sup>, J.M. Massanet<sup>2</sup>, A.A. Reis<sup>2</sup>, H. Rivera<sup>2</sup>, V. Franceschini<sup>2</sup>, A.S. Cornier<sup>2</sup>.* 1) Departemtn of Orthopedics, Mayaguez Medical Center, Mayaguez, PR; 2) Division of Genetics, Ponce School of Medicine, Ponce, PR; 3) Dept. of Nursing, Univ of PR at Arecibo, Arecibo, PR.

Spondylothoracic dysplasia (STD; MIM#277300) is a rare autosomal recessive pleotropic disorder with high prevalence in the Puerto Rican population. Typical findings include segmentation and formation defects throughout cervical, thoracic and lumbar spine such as hemivertebrae, block vertebrae, and unsegmented bars with fusion of all the ribs at the costo-vertebral junction (crab-like chest configuration). It was generally regarded as a lethal condition in the neonatal period due to pneumonia and respiratory failure. Pulmonary hypertension has been documented as well in some patients with STD. We have followed 5 patients, of which 2 was male and 3 were females, ranging from 10 to 22 years old, in our genetics clinic. Pulmonary function tests were performed by a respiratory care technician and supervised by a respiratory medicine specialist and repeated after an interval of 2 to 3 years. The pulmonary function tests showed a severe restrictive pattern with an average FEV1/FVC ratio of 0.98 and 0.90, for the first and second measurements respectively. The average first measurements were: FVC: 23.72 %, FEV1: 26.09 %, PEF: 36.33 %, and the average for the second measurements were: FVC: 27.65 %, FEV1: 28.92 %, PEF: 48.28 %, of the predicted values for the group. Even tough these values tend to be slightly higher in the second series, statistical analysis has not shown the differences to be significant (p values of 0.452 for FVC, 0.618 for FEV1, and 0.351 for PEF). There appears to be some improvement in the pulmonary function of these patients with a decrease in the FEV1/FVC ratio and increased FVC, FEV1, and PEF. Even though these results are not statistically significant, this is probably due to the small sample. However, individually these patients pulmonary function either improved or remained clinically stable as they grew older.

**Long survival in a case of opsismodysplasia.** *P. Strisciuglio<sup>1</sup>, G. Scarano<sup>2</sup>, G.R. Vega<sup>1</sup>, G. Muzzi<sup>1</sup>, M.T. Moricca<sup>1</sup>, R. De Marco<sup>1</sup>, D. Concolino<sup>1</sup>.* 1) Dept Pediatrics, Univ Catanzaro, Catanzaro, Italy; 2) Med. Genet.Division, Hospital of Benevento, Italy.

Opsismodysplasia is a rare chondrodysplasia, first described in 1977 by Zonana et al and designated opsismodysplasia only in 1984 by Maroteaux. This disorder is characterised clinically by micromelia with extremely short hands and feet. Respiratory distress is responsible for death in the first years of life. The main radiological features include severe platyspondyly, delayed skeletal ossification, and metaphyseal cupping. The inheritance seems to be autosomal recessive. We describe the first Italian case of opsismodysplasia with pulmonary hypoplasia and a long survival. S.S. female, at the age of 3 years and 3 months, presented recurrent respiratory infections, and low disharmonic stature. Her weight was of Kg 11,800 (< 5 Centile), the length of 79 cm (<< 5 Centile) , and cranial circumference of 47.3 cm (< 5 Centile). Other important features included large anterior fontanel, short nose with depressed nasal bridge and short hands and feet, coxa vara and metaphyseal cupping of the knees. Molecular analysis of FGFR3 gene did not show mutations in 7,9,10,13, 15 and 19 exons. Until now in literature 13 patients have been described: two of them showed pulmonary hypoplasia and died during the first years of life for respiratory insufficiency. Our patient also has a pulmonary hypoplasia, but he is still in good conditions at the age of 3 years and 7 months. We report this patient with a rare disease, suggesting to take in account the diagnosis of opsismodysplasia in all patients with achondroplasia-like signs and negative molecular analysis and recurrent respiratory infections. Finally the diagnosis of opsismodysplasia is also important for genetic counselling because this syndrome is inherited with autosomal recessive mechanism.

**Hypochondroplasia: Clinical and FGFR3 gene mutation analysis in an Indian pedigree.** *V.K Patel<sup>1</sup>, R. Meda<sup>1</sup>, M.R. Memon<sup>2</sup>, J.V. Solanki<sup>3</sup>, U. Ratnamala<sup>1</sup>, U. Radhakrishna<sup>1</sup>.* 1) RIA Laboratory, Green Cross Voluntary Blood Bank & Genetic centre; 2) Department of Zoology, Gujarat University, Ahmedabad; 3) Department of Animal Genetics & Breeding, Veterinary college, Gujarat Agriculture University, Anand, India.

Hypochondroplasia (HCH; OMIM 146000) in humans is an autosomal dominant skeletal dysplasia characterized by short stature, micromelia, lumbar lordosis, short and broad bones. The skeletal features of hypochondroplasia are similar to that of achondroplasia but milder, and these phenotypes are considered to be allelic. The gene responsible for HCH has been mapped to chromosome 4p16.3 and mutations in the fibroblast growth factor receptor 3 (FGFR3) gene have been identified in patients with the disorder (Nat Genet. 1995 10:357-9). Over 95% of reported cases are accounted by a single Gly380Arg mutation. Some reports suggested HCH is clinically and genetically heterogeneous (J. Med. Genet. 33: 749-752, 1996; J. Med. Genet. 32: 492-493, 1995). We studied a large family from the Western part of India with HCH. The pedigree consists of 42 individuals including 9 affecteds. Clinical findings were recorded for all affecteds and radiographic findings were analyzed in selected individuals. Short stature, micromelia, macrocephaly, short hands and small fingers, shortening of the long bones characterize the phenotype in this family. Genomic DNAs of two affecteds and one normal individual were screened for mutations in the coding region of FGFR3. No mutation was found. Thus this study supports previous data suggesting that HCH be genetically heterogeneous. Systematic genome-wide linkage analyses, using HCH families that are excluded FGFR3 gene mutations may reveal different chromosomal loci for the phenotype. Email: u\_c\_rao@hotmail.com.

**Anatomical and functional brain imaging evidence of lenticulo-insular anomalies in Smith Magenis syndrome.** *N. Boddaert*<sup>1, 2</sup>, *M. Zilbovicius*<sup>2</sup>, *A. Munnich*<sup>3</sup>, *F. Brunelle*<sup>1</sup>, *H. De Leersnyder*<sup>3</sup>. 1) Dept Radiology, Hosp Necker, Paris, France; 2) ERM 0205 INSERM-CEA, Hosp Frederic Joliot, Orsay, France; 3) Dept Ped Genetics, Hosp Necker, Paris, France.

Smith Magenis syndrome (SMS) is a clinically recognizable contiguous gene syndrome ascribed to an interstitial deletion of chromosome 17p11.2. The neurobehavioral phenotype of SMS includes mental retardation, speech delay, hyperactivity, attention deficit, decreased sensitivity to pain, self-injury, aggressive behavior and sleep disturbance. This peculiar phenotype suggests that SMS could be associated with still unknown brain anomalies. Therefore, we performed anatomical and functional brain imaging studies in 5 SMS boys. Anatomical MRI were analyzed using optimized voxel-based morphometry. This method can detect structural anomalies not apparent on visual inspection of the scans. Two comparisons groups were studied: 12 age-matched healthy control children and 5 age-matched children with idiopathic mental retardation. In addition, positron emission tomography and water-labeled method were used to investigate a putative localized brain dysfunction in SMS. The same age-matched mental retarded children composed the control group. A significant bilateral decrease of grey matter concentration was detected in the insula and lenticular nucleus ( $p < 0.0001$ ,  $p < 0.05$  corrected height threshold,  $df = 15$ ) in SMS patients compared to normal children using MRI and optimized voxel-based morphometry. In addition, a significant hypoperfusion was found in the same regions ( $p < 0.001$ ,  $df = 9$ ) detected with PET in SMS patients compared to mentally retarded children. This anatomo-functional evidence of bilateral insulo-lenticular anomalies in SMS should be relevant to neurobehavioral symptoms. In fact, several lines of evidence support the involvement of insula in the complex processes associated with pain and thermosensory control and lenticular nucleus was shown to be involved in attention-deficit/hyperactivity disorder (ADHD). The identification of localized brain anomalies in SMS will hopefully help understanding how this well defined genetic entity can lead to a relatively specific severe neurobehavioral syndrome.

**A clinical study of Mast syndrome, an autosomal recessive form of hereditary spastic paraplegia with dementia.**

*A.H. Crosby<sup>1</sup>, C. Proukakis<sup>1</sup>, M. Simpson<sup>1</sup>, M.A. Patton<sup>1</sup>, H. Cross<sup>2</sup>.* 1) Dept Medical Genetics, St George's Hospital Med Sch, London, United Kingdom SW17 0RE; 2) Dept Ophthalmology, University of Arizona School of Medicine, 655 N. Alveron Way, Tuscon, USA.

Mast syndrome, originally described in 1967, is an autosomal recessive complicated form of hereditary spastic paraplegia (HSP) with dementia present at high frequency amongst the Old Order Amish. We have studied 14 affected individuals with ages ranging from 31 to 62, with a consequent great variation in disease severity. Milestones were sometimes delayed, and mild motor and learning difficulties were often noted in childhood. Several patients were married and had children. Decline in walking and mental function started in early adulthood, although age of onset was difficult to define. The volume of speech also declined and swallowing difficulties arose later. The condition was clearly progressive in all, leading to akinetic mutism in the most severe. Mini mental test scores ranged from 0 to 14. All had clear pyramidal signs which were much more severe in the lower limbs. Mild cerebellar abnormalities were seen, and the most advanced cases also had extrapyramidal movements. MRI scans in 3 patients revealed a thin corpus callosum, cerebral atrophy and white matter abnormalities. The Mast syndrome is thus an example of HSP associated with adult-onset dementia and a thin corpus callosum. Parallel genetic studies have led to the identification of a causative mutation in the polypeptide product of SPG21, designated maspardin (Mast syndrome, spastic paraplegia, autosomal recessive with dementia).

**Fetal brain malformation with pseudo-infectious features in three successive pregnancies.** *R.J.M. Gardner, S.M. White.* Genetic Health Services Victoria, Monash Medical Centre and Royal Children's Hospital, Melbourne, Australia.

Aicardi-Goutieres and Pseudo-TORCH syndromes are recessively inherited disorders of brain malformation in which the appearance somewhat resembles an intrauterine infection. We have seen a condition which may also warrant inclusion in this category, in which the brain malformation is more severe than with either of these two diagnoses. All three pregnancies (one female, two male) of a nonconsanguineous Anglo-Celtic couple were identified on ultrasonography with severe underdevelopment of the brain by 17-19 weeks (earlier scans having been apparently normal), with terminations done at 18-19 weeks. In the third pregnancy, fetal MRI demonstrated the anatomy of the defect with clarity at 19 weeks gestation. Pathology studies showed severe thinning of the parenchyma of the cerebral hemispheres (3-4 mm), and hypoplasia of the cerebellar hemispheres and deficiency of the vermis. On histology, severe cerebral leukomalacia, and multiple foci of calcification but without inflammatory infiltration, were noted. We propose that these cases represent a new recessively inherited syndrome of global brain maldevelopment with a pseudo-infectious histopathology.

**Familial isolated congenital anosmia with morphologically normal olfactory bulb in two unrelated Iranian families.** *M. Ghadami*<sup>1,4,5</sup>, *K. Majidzadeh-A*<sup>6</sup>, *K.S. Morovvati*<sup>6</sup>, *E. Damavandi*<sup>2</sup>, *G. Nishimura*<sup>3</sup>, *N. Niikawa*<sup>1,4</sup>, *K.I. Yoshiura*<sup>1,4</sup>, *Mohsen Ghadami*. 1) Dept Human Genetics, Nagasaki Univ Sch Med, Nagasaki, Japan; 2) Department of Cell Biology and Histology, Nagasaki University; 3) Tokyo Metropolitan Kiyose Children's Hospital; 4) Japan Science and Technology Corporation, Japan; 5) Department of Medical Genetics, Tehran University of Medical Sciences, Iran; 6) Jihad Research Center, Tehran University of Medical Sciences, Iran.

Congenital total loss of the sense of smell occurs as a part of a syndrome, e.g., Kallmann syndrome or an isolated form. Congenital isolated anosmia (MIM 107200) is very rare, and only a few families with the disease have been reported to date. It appears to be due to a change in the olfactory epithelium or aplasia of the olfactory nerve, bulb and tract. Here we report two unrelated Iranian families with isolated congenital anosmia. One family consisted of a total of 9 affected members, and the other family contained 3 affected members. Anosmia in each affected member was confirmed by clinical history, physical examinations and smell testing by intravenous injection of combined vitamins (Arinamin<sup>TM</sup>, Takeda Pharmaceutical Co. Ltd., Japan). No signs of hypogonadism or other neurological disorders was observed in any affected members. Family analysis with the complete ascertainment method under assumption of the same condition in the two families suggested that the disease is not inconsistent with an autosomal dominant mode with incomplete penetrance. The inheritance in one family appears unusual, as if it shows a reverse anticipation pattern, i.e., the lower generation the less number of affected persons. When only the upper two generations in the family are concerned, the segregation ratio was 0.39 0.11. Male-to-male transmissions and affectedness of both sexes were observed in both families. Magnetic resonance imaging (MRI) of the olfactory bulb and sulcus revealed no evidence of morphological changes in affected members, suggesting that these patients have a functional defect in the olfactory system, and the condition in the two families is a new type of anosmia.

**Hypocalcemia and the neuropsychiatric phenotype of 22q11.2 Deletion Syndrome.** *A.S. Bassett<sup>1,2</sup>, O. Caluseriu<sup>2,3</sup>, V. Wong<sup>2</sup>, M. Gheroghiu<sup>2</sup>, R. Weksberg<sup>1,3</sup>, E.W.C. Chow<sup>1,2</sup>.* 1) University of Toronto, Toronto, ON, Canada; 2) Centre for Addiction & Mental Health; 3) Hospital for Sick Children.

22q11.2 Deletion Syndrome (22qDS) is associated with a microdeletion on chromosome 22q11.2 and variable multisystem features. The central nervous system (CNS) is commonly involved with learning disabilities and psychiatric illness like schizophrenia presenting over time. Hypocalcemia and hypoparathyroidism are reported in infants and children with 22qDS, usually as transient conditions. We investigated rates of lifetime hypocalcemia in 63 (27 M; 36 F) adults with 22qDS and its association with major CNS features. 46 (73.0%) 22qDS subjects (median age 28 y) had a history of hypocalcemia, first detected in infancy (n=10), at paediatric surgery (n=2), later in childhood (n=7) or on systematic testing as adults through our clinic (n=27, median age 27). Parathyroid hormone levels were inappropriately low in all but 4 subjects with hypocalcemia. There was a high risk of seizures in subjects with hypocalcemia. Rates of hypocalcemia were similar in 33 subjects with schizophrenia (22qDS-SZ; 75.8%) and 30 with no psychosis (22qDS-NP; 70.0%) (p=0.61). Hypocalcemia had no effect on IQ or age at onset of schizophrenia in the 22qDS-SZ group. However, subjects with a history of hypocalcemia in the 22qDS-NP group had significantly lower mean full scale IQ than those without hypocalcemia (n=17, 68.5 SD 9.4 vs. N=7, 78.3 SD 6.1, p=0.02). Hypocalcemia is prevalent in adults with 22qDS and may be associated with significant CNS morbidity. Aggressive monitoring for and treatment of hypocalcemia in 22qDS is warranted across the lifespan.

**Neuroaxonal dystrophy in partial trisomy 5 and monosomy 15q of maternal origin.** *D. McFadden, P. MacLeod, G. Jevon, K. Neuert, M. Parslow, G. Hendson.* Dept Pathology and Medical Genetics, University of British Columbia, Vancouver, BC.

An unbalanced translocation, 46, XX, der(5)t(5;15)(q13;q13)mat, resulting in trisomy for the short arm and proximal long arm of chromosome 5 and monosomy for the proximal long arm of chromosome 15 was diagnosed prenatally after detection of growth restriction. FISH with a SNRPN probe showed no hybridization to der(5), indicating monosomy for SNRPN, with loss of the maternal allele as seen in Angelman syndrome (AS). The child was dysmorphic and had a laryngeal web, tracheomalacia, and an atrial septal defect (ASD). She died at 9 weeks of respiratory failure.

At autopsy, dysmorphic features included cranial asymmetry, broad nasal bridge, anteverted nares, short upslanting palpebral fissures, prominent infraorbital creases, large fontanelles, and an ASD.

The most striking finding was of neuroaxonal dystrophy with axonal spheroids throughout the grey matter of the central nervous system (CNS). These were not associated with iron deposition and the cerebellum was of normal size. Mild ventriculomegaly and changes of hypoxic-ischemic encephalopathy were present.

Trisomy 5p is characterized by macrocephaly, facial dysmorphisms, cardiac and renal anomalies, and abnormal CNS with ventriculomegaly and periventricular heterotopic neuroglial tissue. Duplications of proximal 5q are uncommon; the phenotype is characterized by developmental delay without other anomalies. The phenotype of AS is well known. Neuropathologic study is reported in two cases of AS and has documented cerebral atrophy and variable cerebellar hypoplasia.

Neuroaxonal dystrophy is characteristic of inherited neuroaxonal dystrophies. This case differs from these in that the spheroids were not associated with cerebellar atrophy or iron deposition. CNS neuroaxonal dystrophy is uncommon outside the setting of the primary neuroaxonal dystrophies and has not been previously reported in chromosome abnormalities. This may represent a new finding associated with deletion of proximal 15q of maternal origin.

**Down syndrome mouse models Ts65Dn, Ts1Cje, and Ms1Cje/Ts65Dn exhibit variable severity of cerebellar phenotypes.** *L.E. Olson<sup>1</sup>, L.L. Baxter<sup>2</sup>, E.J. Carlson<sup>3</sup>, C.J. Epstein<sup>3</sup>, R.H. Reeves<sup>1</sup>*. 1) Department of Physiology, Johns Hopkins School of Medicine, Baltimore, MD; 2) National Institutes of Health, National Human Genome Research Institute, Bethesda, MD; 3) Department of Pediatrics, University of California, San Francisco, CA.

The mechanisms by which trisomy 21 causes specific traits of Down syndrome (DS) are unknown. One qualitative hypothesis proposes that small regions of chromosome 21 are sufficient to cause specific phenotypes of DS, while the quantitative hypothesis asserts that defects occur due to small effects of many genes. We evaluated the cerebellar phenotype of DS using mouse models that are trisomic for different regions orthologous to human chromosome 21. The Ts65Dn mouse is trisomic for approximately 113 genes; complementary subsets of 89 and 23 of these genes are trisomic in the Ts1Cje and Ms1Cje/Ts65Dn mouse models, respectively. Using high-resolution 3D MRI and histological analyses, we calculated total cerebellar volume and cell densities of granule cells and Purkinje cells in Ts1Cje and Ms1Cje/Ts65Dn mice. By comparing our analysis to a previous study of the Ts65Dn mouse (Baxter et al., *Hum Mol Genet*, 2000), we show that cerebellar volume was reduced to the same extent in Ts65Dn and Ts1Cje mice but not reduced at all in the Ms1Cje/Ts65Dn model. Granule cell density was equally affected in both Ts1Cje model and Ms1Cje/Ts65Dn, but to a lesser degree than in Ts65Dn. Purkinje cell density, which is significantly reduced in Ts65Dn mice, was unaffected in either Ts1Cje or Ms1Cje/Ts65Dn mice. Neither the qualitative nor the quantitative hypothesis makes a prediction to cover the various outcomes seen here. A more useful description, therefore, is whether a region of trisomy is necessary and/or sufficient to cause a phenotype. The region of trisomy sufficient to cause cerebellar reduction has been narrowed to the Ts1Cje region; the Ts1Cje and the Ms1Cje/Ts65Dn segments may have an additive affect to produce the degree of granule cell deficiency seen in Ts65Dn; and neither of these regions is sufficient to cause Purkinje cell density reduction.

**The Floating-Harbor syndrome: Is it really that rare?** *C. Morel<sup>1,2</sup>, L. Tidmarsh<sup>3</sup>, C. Poulin<sup>4</sup>, M. Sufrategui<sup>5</sup>, V. Der Kaloustian<sup>1,2</sup>*. 1) Dept of Human Genetics, McGill University, Montreal, QC, Canada; 2) Dept of Medical Genetics, Montreal Children's Hospital, Montreal, QC, Canada; 3) Dept of Psychiatry, Montreal Children's Hospital, Montreal, QC, Canada; 4) Dept of Neurology and Neurosurgery, Montreal Children's Hospital, Montreal, QC, Canada; 5) Dept of Psychology, Montreal Children's Hospital, Montreal, QC, Canada.

The Floating-Harbour Syndrome is a rare condition presenting with short stature, delayed bone age and expressive language delay. The facial features characteristic for this syndrome are a triangular face with a broad nasal bridge and deep-set eyes. We present a 6-year-old boy with the clinical picture of the Floating-Harbour syndrome, but with cerebral MRI findings previously undescribed in the literature. Our patient was born after a normal pregnancy and by induced vaginal delivery. Up until the age of 3, he had irregular sleeping patterns. Acquisition of developmental milestones was globally delayed. His cognitive development was evaluated and revealed mild mental deficiency at the age of 5. The sphere of development the most severely impaired was that of language, with expressive language problems predominating. In addition, he presented with severe oppositional behaviour. Physical examination revealed a height, weight and head circumference within normal limits. He has a triangular face, long eyelashes, a broad-based nose, wide columella, broad mouth, thin lips, low-set and posteriorly-angulated ears. His voice is high-pitched and nasal, and he also has 5th finger clinodactyly. The following examinations revealed normal results: Fragile-X test, blood karyotype, EKG and echocardiogram. Audiologic examination suggested a mild conductive hearing loss. EEG showed occasional epileptiform activity during wakefulness, drowsiness and sleep. Cerebral MRI studies revealed asymmetry of the lateral ventricles with a larger left ventricle and mildly abnormal development of the left cerebral hemisphere. Bone age was delayed by 1.5 years. Our patient represents the 31st case described with the diagnosis of Floating-Harbour syndrome. The results of some of his neurological testing may help to better delineate this rare syndrome.

**Central nervous system malformations in a child with Knobloch (encephalocele-myopia-retinal detachment) syndrome.** *B. Keren*<sup>1</sup>, *A. Monnier*<sup>2</sup>, *N. Blanc*<sup>2</sup>, *M. Elmaleh*<sup>3</sup>, *D. Bremond*<sup>4</sup>, *C. Baumann*<sup>1</sup>, *A. Verloes*<sup>1,5</sup>. 1) Clinical Genetic Unit; 2) Pediatric Neurology Dept; 3) Medical Imaging Dept; 4) Ophthalmology Dept; 5) INSERM E9935, Hôpital Robert Debré Paris, France.

Knobloch syndrome is a combination of posterior midline encephalocele, macular abnormalities, high grade myopia, vitreoretinal degeneration with retinal detachment and normal intelligence, suggesting alterations during early neuroectodermal morphogenesis. 28 cases have been reported insofar. It has been shown to be due to mutations in COL18A1 gene, mapped to 21q22.3. It leads to abnormal expression of collagen XVIII and endostatin. Endostatin is the non triplehelical C-terminal NC1 globular domain of collagen XVIII. This proteolytically derived component is located in almost all epithelial basement membranes of capillaries and blood vessels, and in basement membranes of all major developing organs. Endostatin controls neuronal guidance in *Caenorhabditis. elegans*. Abnormal neuronal migration has been very recently reported in Knobloch syndrome by Kliemann et al. (*Am J Med Genet.* 2003, 119A:15-9). We report a 3 year-old girl born to consanguineous Maghrebian parents with high grade myopia (-15d), perimacular retinal coloboma, septo-optic dysplasia (septal agenesis and optic nerve hypoplasia), symmetric frontal micropolygyria, occipital meningocele and subnormal psychomotor development. This patient expand the range of CNS malformations observed with Knobloch syndrome and confirms the implication of endostatin in neuronal migration and CNS organization in higher vertebrates. Screening for mutations in COL18A1 is pending.

**Idiopathic Seizures In Patients With A 22q11.2 Deletion.** A. Kao, D.M. McDonald-McGinn, J. Mariani, M.K. Maisenbacher, E.H. Zackai, D.R. Lynch. The Children's Hosp of Phila Philadelphia, PA.

Brain anomalies have been described in the 22q11.2 deletion including underopercularization & polymicrogyria(Bingham, 1997 & 1998), cerebellar hypoplasia(Lynch 1995), atrophy(Eliez 2000) & agenesis of the corpus callosum(Ryan 1997 & McDonald-McGinn 1999). Other neurologic/cognitive features include: hypotonia, microcephaly, asymmetric crying facies, ADD, & nonverbal learning disability(Moss 1999). Further there are isolated reports of recurrent unprovoked seizures. Whether these are coincidental, secondary or primary is unclear. We therefore reviewed the records of 348 deleted CHOP patients for documentation of seizures including EEG findings & causal events (hypocalcemia, fever, recent surgery). 94% were 20y or younger (mean 9.5y & median 8y). 81(23%) had seizures including 55(16%) with provoked seizures (hypocalcemia, post-op complications, hypoxic/ishemic events, fever). However, 26(7.5%) had unprovoked seizures w/o clear precipitating events, 19 with focal & 7 with primary generalized seizures (PGS). Most focal patients had onset by 4y. PGS patients had onset as neonates(2) or as adolescence(5). 5 PGS patients had MRIs-all normal. 10 focal patients had MRI abnormalities such as polymicrogyria(2), focal cortical dysplasia, unspecified cortical nodules, cerebellar degeneration, hypomyelination, temporal edema, & diffuse cortical atrophy suggestive of epilepsy due to a brain lesion. So excluding these 10 patients, 16(4.6%) deleted patients had idiopathic or generalized seizures, in contrast to the general population incidence of 2.8 to 20.7/1000 (Hauser 2001). In addition we found that cardiac disease, family history, & prematurity were not risk factors for the development of unprovoked seizures (Fishers exact test). Of note, recent linkage studies have mapped an epilepsy locus to 22q11-12 (Xiong 1999). Thus our findings suggest that the idiopathic seizures found in our cohort represent a primary manifestation of the deletion. Furthermore, these results have implications for consideration of the 22q11.2 deletion in patients presenting primarily for epilepsy and for the identification of potential genetic loci responsible for idiopathic epilepsy.

**Fragile X-associated tremor/ataxia syndrome (FXTAS) involvement in females.** *R.J. Hagerman<sup>1</sup>, S. Jacquemont<sup>1</sup>, F. Tassone<sup>2</sup>, C. Greco<sup>3</sup>, J. Brunberg<sup>4</sup>, D. Hessel<sup>1</sup>, S. Harris<sup>1</sup>, L. Zhang<sup>5</sup>, T. Jardini<sup>1</sup>, L. Ruiz<sup>1</sup>, L. Gane<sup>1</sup>, P.J. Hagerman<sup>2</sup>.* 1) MIND Institute, Univ. of California Davis Med. Center, Sacramento, CA; 2) Biological Chemistry, Univ. of California Davis School of Med., Davis, CA; 3) Pathology, Univ. of California Davis Med. Center, Sacramento, CA; 4) Radiology, Univ. of California Davis Med. Center, Sacramento, CA; 5) Neurology, Univ. of California Davis Med. Center, Sacramento, CA.

FXTAS is a progressive neurological disorder that affects some older male carriers (50 yo) of premutation alleles of the *FMR1* gene. Presentation typically involves intention tremor and/or gait ataxia, parkinsonism, autonomic dysfunction; often accompanied by cognitive deficits and subsequent dementia. MRI findings include symmetric hyperintensities in the middle cerebellar peduncles (MCP sign). Neuropathologic changes include intranuclear (neuronal and astrocytic) inclusions. Previous published reports have not identified FXTAS in females. We report 3 females with premutation alleles who present with clinical features of FXTAS, yet none shows cognitive involvement seen in males with FXTAS. Case 1 is a 67 yo female [full scale IQ, 126; 90 CGG repeats; mRNA level, 3.250.55, FMRP level, 89%; activation ratio (AR), 0.5], with gradually progressive intention tremor (35 yrs) and ataxia (12 yrs). T2-weighted MRI demonstrates mild atrophy with symmetric hyperintensities of the MCPs. Case 2 is the 62yo sister of Case 1 with a history of intention tremor (10 yrs) and ataxia with frequent falling (2 yrs), but no executive function deficits [full scale IQ, 111; 90 CGG repeats; mRNA level, 2.520.27; AR, 0.50; FMRP, 70%]. Case 3 is deceased an 85 yo female [87 repeats; AR 0.6] who had mild tremor (3 yrs), and mild ataxia complicated by hip surgery, eventually leading to cane use at 79y. She had significant anxiety without cognitive impairment (full scale IQ of 100). Upon autopsy, neuropathologic examination revealed mild atrophy of the posterior frontal region and intranuclear inclusions in the hippocampus. FXTAS occurs infrequently in females, who also appear to be relatively protected from the cognitive deficits associated with FXTAS.

### **Structural Neuroimaging and Molecular Correlates of Psychopathology in Adult Males with the FMR1**

**Premutation.** D. Hessl<sup>1</sup>, C. Cohen<sup>1</sup>, C. DeCarlie<sup>3</sup>, R. Tong-Turnbeaugh<sup>3</sup>, S. Jacquemont<sup>1</sup>, L. Gane<sup>1</sup>, T. Jardini<sup>1</sup>, J. Wegelin<sup>4</sup>, F. Tassone<sup>2</sup>, P.J. Hagerman<sup>2</sup>, R.J. Hagerman<sup>1</sup>. 1) M.I.N.D. Institute, U.C. Davis, Sacramento, CA; 2) Biological Chemistry, U.C. Davis, Davis, CA; 3) Neurology, U.C. Davis, Sacramento, CA; 4) Epidemiology, U.C. Davis, Davis, CA.

**BACKGROUND:** Fragile X associated tremor/ataxia syndrome (FXTAS) is a recently described disorder in of male carriers of premutation alleles of the fragile X mental retardation 1 (*FMR1*) gene. FXTAS is characterized by gait ataxia and/or intention tremor and Parkinsonism. Brain MRI has revealed white matter signal hyperintensity in the middle cerebellar peduncles, cerebellar atrophy, and intranuclear inclusions throughout the cortex and brain stem, with highest concentration in the hippocampus. Psychopathology associated with FXTAS has not been previously described.

**METHOD:** Sixteen premutation males ( $M = 61$  yrs) and male controls ( $M = 67$  yrs) underwent brain MRI, FMR1 protein and mRNA analysis, neurological examination, and psychological assessment (SCL-90-R). Cerebellar and hippocampal volumes, corrected for cranial size, were compared and correlated with SCL-90-R scores and *FMR1* molecular measures. **RESULTS:** MRI analysis showed reduced cerebellar volume in premutation subjects compared to controls that approached significance ( $t = 1.87$ ,  $p = .07$ ), but no significant differences in hippocampal or cerebral volume were found. Within the premutation group only, reduced hippocampal volume was significantly associated with increased psychopathology,  $r = -.81$ ,  $p = .005$ , especially symptoms of depression, interpersonal sensitivity, and psychoticism. This association remained significant after adjusting for severity of neurological impairment through standardized measures of tremor and ataxia. Increased FMR1 mRNA and *FMR1* protein were positively correlated with psychopathology,  $r_s = .70$  and  $.77$  respectively,  $p < .05$ , such that increased message and protein were associated with increased psychopathology. **CONCLUSION:** Molecular alterations due to the *FMR1* premutation in those affected by FXTAS may lead to increased psychological symptoms as a result of neuropathology in the hippocampus that are independent of tremor and ataxia.

**Trisomy 2p and monosomy 8p in a patient with DiGeorge syndrome.** *R. Stephane<sup>1</sup>, D. Genevieve<sup>1</sup>, D. Sanlaville<sup>1</sup>, A. Maltret<sup>1</sup>, S. Sererro<sup>2</sup>, C. Beyler<sup>3</sup>, V. Cormier-Daire<sup>1</sup>, M. Vekemans<sup>1</sup>, A. Munnich<sup>1</sup>, S. Lyonnet<sup>1</sup>, D. Bonnet<sup>3</sup>.* 1) Department of genetics, Hopital Necker-Enfants Malades, Paris; 2) Cytogenetics Unit, Evreux; 3) Cardiology Unit, Hopital Necker-Enfants Malades, Paris, France.

Microdeletion 22q11.23 is the most frequent chromosomal anomaly in syndromic conotruncal heart defect (CTHD). However, other cytogenetic anomalies have been reported in CTHD including deletion of chromosome 10p13-14 and 4q34.2. Here, we report on a patient with pulmonary atresia and ventricular septal defect with partial trisomy 2p22-pter and partial monosomy 8p23-pter. The balanced 46,XY, t (2;8)(p22; p23) translocation, was found to be carried by the healthy father. Clinical features were consistent with DiGeorge syndrome namely facial dysmorphism, arachnodactyly, thymic hypoplasia and hypocalcemia. In addition, skeletal X-rays showed absent distal phalanges on both feet, sacral agenesis and calcaneal punctuations. Abdominal CT scan showed a right adrenal gland mass which was diagnosed as a neuroblastoma. FISH analysis ruled out chromosome 22q11.2 deletion. So far, 85% of patients reported with a terminal deletion 8p presented with heart defects, of which 27% were CTHD. Other features were minor facial dysmorphic features, growth retardation, microcephaly, developmental delay and genitourinary anomalies. On the other hand, patients with trisomy 2p present with facial dysmorphism including small palpebral fissures, enlarged nasal bones, small mouth, arachnodactyly, psychomotor delay and congenital heart defect. Interestingly, these patients also show a predisposition to neuroblastoma. Therefore, the facial features observed in our patient could be the result of the combination of the overlapping features ascribed to monosomy 8p and trisomy 2p. On the other hand, we suggest that neuroblastoma may result from the duplication of either the proto-oncogene N-myc or the NAG gene mapping to 2p23.4 and 2p25.1 respectively. We propose that all patients with atypical DiGeorge phenotype and normal 22q11.2 FISH should be carefully investigated for both deletion of 8p23.1 and trisomy of 2p, especially as a predisposition to neuroblastoma may be involved.

**X-inactivation Analysis in female carriers of ATR-X (X-linked -thalassemia/mental retardation syndrome).** T. Wada<sup>1</sup>, A. Sudo<sup>2</sup>, Y. Fukushima<sup>1</sup>, S. Saitoh<sup>2</sup>. 1) Shinshu Univ Sch of Medicine, Matsumoto, Japan; 2) Hokkaido Univ Sch of Medicine, Sapporo, Japan.

**Introduction** ATR-X (MIM#301040) is among syndromic forms of X-linked mental retardation, and is characterized with severe mental retardation, mild HbH disease, dysmorphic facies, genital abnormalities, skeletal abnormalities, and behavioral abnormalities. Mutations of *ATRX* give rise to ATR-X. Thus far, 26 patients from 23 families in Japan have been diagnosed as ATR-X. We reported here the X-inactivation analysis in female families of ATR-X patients.

**Subjects and Methods** Twenty HbH positive cases from 18 families and 14 HbH negative cases from 14 families who demonstrated characteristic features for ATR-X were examined for *ATRX* mutations. We performed the X-inactivation analysis for female family members whose DNA was available. *ATRX* mutations were screened by RT-PCR and direct sequencing using lymphoblastoid cells, and confirmed using genomic DNA. For X-inactivation analysis, genomic DNA was digested with a methylation-sensitive restriction enzyme, and subsequently the polymorphic CAG repeats in the *HUMARA* gene were PCR amplified. **Results** We detected *ATRX* mutations in 18 HbH positive cases from 16 families and 7 HbH negative cases from 7 families. Nine out of 11 mothers (81.8%) of the ATR-X patients were carriers. We analyzed the X-inactivation pattern for 9 females from 7 families. Three out of nine females were excluded, because their CAG repeats of *HUMARA* gene were homozygous and we could not differentiate two alleles. In 4 female carriers who had a mutation, three showed a skewed pattern but one showed a not-skewed pattern (70: 30), and she had mild mental retardation. Two females without mutations showed a random pattern. **Discussion** Females carriers with *ATRX* mutations have been reported to have no mental retardation, because the X chromosome with an *ATRX* mutation is selectively inactivated. Our case of the female with mild MR may indicate that an *ATRX* mutation could cause mental retardation in female, if skewed X-inactivation is not strictly maintained. We should study more families to confirm this hypothesis, and we are analyzing another family with a mild MR female.

**Baraitser-Winter (coloboma-ptosis-pachygyria) syndrome.** A. Verloes<sup>1,2</sup>, C. Baumann<sup>1</sup>, P. Blanchet<sup>3</sup>, A. David<sup>4</sup>, J. Roume<sup>5</sup>, C. Rusu<sup>6</sup>, P. Sarda<sup>3</sup>, M. Till<sup>7</sup>, D. Pilz<sup>8</sup>. 1) Clinical Genetics Unit; 2) and INSERM E9935, Hosp Robert DEBRE, Paris; 3) Genetic dept, Hosp A. de Villeneuve, Montpellier; 4) Genetic Dept, University Hospital, Nantes; 5) Genetic Unit, CH Intercommunal, Clinical Genetics Unit, CH Intercommunal, Poissy - Saint Germain-en-Laye, France; 6) Genetic Dept, Iasi University Hospital, Romania; 7) Genetic Dept, Hpital Debrousse, Lyon, France; 8) Genetic Dept, University Hospital of Wales, Cardiff, UK.

Baraitser-Winter syndrome (BW) is an exceptional disorder (5 patients reported) characterized by short stature, hypertelorism, broad epicanthus, bilateral ptosis, coloboma, metopic ridge and pachygyria. Fryns-Aftimos syndrome (FA), reported in 9 patients, is defined by hypertelorism, ptosis, large nose, neck webbing, low posterior hairline, broad thorax, frontal pachygyria, preaxial anomalies and seizures. An unusual body habitus is present, with truncal obesity, tip-toe walking, limited extension of knees and shoulder propulsion. Both disorders are reminiscent of Noonan syndrome and both are associated with mild to severe mental retardation. We report a series of 8 patients (some followed for more than 10 years) with a phenotype overlapping BW syndrome and FA syndrome, suggesting that both disorders are identical (but reported at different ages : youngsters in BW, teen-agers in FA), and possibly less exceptional than previously thought. PTPN11 screen was negative in 3 of our cases, and telomere screen in several of them. Facial Gestalt of BW seems the most reliable clue for diagnosis. Predominantly frontal pachygyria and coloboma are common but inconstant. Mental retardation is variable and may be influenced by epilepsy. Contrasting with Noonan syndrome, heart is not a target. Most cases are sporadic. Based on this new series, we propose a renewed definition of Baraitser-Winter syndrome and will review the earlier literature.

**Two distinct mutations are responsible for the higher prevalence of Hereditary Sensory and Autonomic Neuropathy Type II (HSAN II) in French Canadians.** *K. Roddier<sup>1</sup>, T. Thomas<sup>1</sup>, G. Marleau<sup>1</sup>, A.M. Gagnon<sup>1</sup>, M.J. Dicaire<sup>1</sup>, A. Saint-Denis<sup>1</sup>, A.M. Sarrazin<sup>2</sup>, A. Larbrisseau<sup>3</sup>, M. Lambert<sup>3</sup>, M. Vanasse<sup>3</sup>, M. Samuels<sup>4</sup>, G.A. Rouleau<sup>4</sup>, B. Brais<sup>1</sup>.* 1) CHUM, Université de Montréal, Montreal, Quebec, Canada; 2) 2. Centre Hospitalier Régional de Lanaudière, Joliette, Quebec, Canada; 3) 3. Hôpital Ste-Justine, Montréal, Quebec, Canada; 4) 4. Xenon Genetics Inc., Burnaby, B.C. Canada.

Hereditary sensory and autonomic neuropathy type II (HSAN II, OMIM 201300) is a rare childhood recessive neuropathy. It was first clearly described in 1973 in a French-Canadian (FC) kinship. The HSANII locus and the *HSN2* mutated gene have been uncovered. Purpose. Establish that a higher prevalence of this condition exists in the FC population. Fine-map the candidate interval using linkage disequilibrium analysis. Uncover the number of mutations responsible for FC HSANII cases. Methods. We recruited all cases that were evaluated in pediatric and neurological clinics during the past 30 years. Haplotypes were constructed for all carrier chromosomes by genotyping nine polymorphic markers. The *HSN2* gene was sequenced. Results. We have uncovered the largest cluster ever described of 17 HSANII cases belonging to 12 families. They all originated from southern Quebec. Haplotype analysis determined that two distinct mutations were responsible for HSANII in FC. Linkage disequilibrium analysis fine-mapped the candidate region to a 1cM (225Kbp) interval. Two distinct loss-of-function *HSN2* mutations were identified. The higher prevalence of this condition in Quebec appears to be due to a relatively high carrier frequencies for both mutations. The following genotypes were uncovered in the different families: seven (58.3%) cases were homozygote for mutation 1, one (8.3%) was homozygote for mutation 2 and four (33.3%) were compound heterozygotes for the two mutations. 75% of chromosomes carried mutation 1 and 25% mutation 2. Conclusion. HSANII is more frequent in the FC population due to higher carrier rates for two distinct *HSN2* mutations. This is the first example of a FC recessive disease where the most common mutation is responsible for less than 90% of causal mutations.

**Keratosis Palmaris et Plantaris with Plagiocephaly.** *J.C. Prieto<sup>1,2</sup>, H. Velasco<sup>1</sup>*. 1) Instituto de Genetica Humana, Universidad Javeriana, Bogota, Cundinamarca, Colombia; 2) Hospital la Victoria, SDS, Bogota, Colombia.

The inherited palmoplantar keratodermas are an extremely heterogeneous group of skin diseases that can be subclassified on the basis of the pattern and age of onset of hyperkeratosis on the palm and sole and on whether they fall into clinical syndromes and, much more recently, by their inherited genetic defect. Keratosis palmaris et plantaris is an autosomal dominant disorder in which hyperkeratosis confined to the palms and soles is characterized histologically by cytolysis of suprabasal keratinocytes which usually becomes evident between the ages of 3 and 12 months. The underlying defects of some genodermatoses of the epidermis that involve mutations of the keratin proteins have been found. Mutations in the keratin 9 gene (KRT9), a type I keratin expressed exclusively in the suprabasal keratinocytes of palmoplantar epidermis, have previously been demonstrated in this disorder. Others forms the keratosis palmaris can include clinodactyly or deafness. Here we report on 2 year old boy with normal stature, plagiocephaly, hyperkeratosis palmo-plantar, rocker-bottom feet, and vertical talus. The cytogenetic analysis was normal 46,XY. The CT images showed plagiocephaly. We present this case of Keratosis palmaris et plantaris with thirteen affect family members in which is demonstrated the autosomal dominant inheritance pattern and intrafamilial phenotypic variability. To our knowledge, not other reports have described patients with this disease and plagiocephaly and rocker-bottom feet. This new case contributes with new clinic and radiological findings that have not been reported on previous cases.

**The spectrum of conditions with the molar tooth sign: more than just Joubert syndrome.** *M.A. Parisi<sup>1</sup>, C.L. Bennett<sup>1</sup>, W.B. Dobyns<sup>2</sup>, B.L. Maria<sup>3</sup>, P.F. Chance<sup>1</sup>, I.A. Glass<sup>1</sup>.* 1) Dept of Pediatrics, Univ of Washington, Seattle, WA; 2) Dept of Human Genetics, Univ of Chicago, Chicago, IL; 3) Dept of Child Health, Univ of Missouri, Columbia, MO.

Joubert syndrome (JS) is an autosomal recessive condition characterized by hypotonia, neonatal breathing abnormalities, ataxia, developmental delays, and abnormal eye movements known as oculomotor apraxia. MRI changes in JS include cerebellar vermis hypoplasia with distinctive brainstem abnormalities comprising the molar tooth sign (MTS), proposed to be pathognomonic for JS. Variable features include postaxial polydactyly, retinal dystrophy, ocular colobomas, renal complications that include cystic dysplastic kidneys (CDK) or nephronophthisis (NPHP), and hepatic fibrosis. The MTS has been identified in individuals with Arima, Senior-Loken, and COACH syndromes in the past. Here we summarize our experience with 74 JS pedigrees and describe the clinical features in the probands. We extend the spectrum of conditions with the MTS to include individuals with Oral-Facial-Digital syndrome type VI (OFD VI), and provide further evidence of the heterogeneity of conditions with the MTS. Of the 74 pedigrees, 96% have cerebellar vermis hypoplasia while 65% have a documented MTS. 73% have abnormal eye movements, and 68% have a history of breathing abnormalities. In contrast to previous reports of a low incidence of polydactyly, 20% of our families have this feature. Of the 22% with kidney involvement, 4 have CDK and 2 have NPHP. Four are reported to have liver involvement, with one demonstrating the features of COACH syndrome. Two siblings have mesaxial polydactyly and lingual papules suggestive of OFD VI. Although the genetic causes of JS have not been identified, two consanguineous Arab pedigrees showed linkage to 9q34. This locus has been excluded in several families, establishing the genetic heterogeneity of JS. We suggest that clinical delineation of the findings in the MTS syndromes will allow subcategorization to aid in genetic linkage studies. We propose the term Joubert syndrome and related disorders to encompass these conditions that share the MTS but have significant clinical differences.

**Four neurofibroma phenotypes in NF1: The intersection of clinical acumen, natural history and histopathology.**  
*V. Riccardi.* The Neurofibromatosis Institute, La Crescenta, CA.

There are at least four types of neurofibromas appreciated from clinical and natural history vantage points, while histopathologists simply refer to neurofibroma, as though there were not multiple types. These differences in terms of clinical acumen and natural history on the one hand and histopathology, on the other, are critical to devising studies to test treatment strategies and investigate pathogenesis. Analyses of neurofibromas from over 1,000 patients with NF1 in the combined Baylor NF Program and Neurofibromatosis Institute data sets indicate that there are four types of neurofibromas in NF1 in terms of their topography and gross anatomy, time of appearance, rate of growth and associated consequences and complications, including overall severity and mortality. **Cutaneous neurofibroma:** Located in the skin with sparing of certain areas (shin, glans penis), derived from minor nerves; soft, blend into adjacent tissue and non-tender; non-congenital; slow-growing, but may number in the thousands; cosmesis, excessive itching and compromised wearing of clothing. **Subcutaneous neurofibroma:** Located under the skin, derived from medium-sized nerves; firm, discrete and tender; non-congenital; moderate rate of growth; early onset is harbinger of serious nodular plexiform neurofibromas and their attendant compromises. **Nodular plexiform neurofibroma:** Located on dorsal nerve roots, plexuses and large nerves; firm, twisted-rope appearance, confined by perineurium, painful and tender; non-congenital; moderate to fast rate of growth, with spinal cord and peripheral nerve compromises including paralysis, severe pain, neurofibrosarcoma and premature death. **Diffuse plexiform neurofibroma:** Located in the skin and/or internally (eg, mediastinum), soft and fleshy, may have overlying hyper pigmentation and hypertrichosis, invades local tissue beyond perineurium, generally not tender or painful; congenital; slow, erratic rate of growth, with potential of massive size leading to crowding out of local viscera, loss of extremity use and neurofibrosarcoma development and premature death.

**An autosomal dominant hidradenitis suppurativa in a large Indian family.** *U. Radhakrishna<sup>1</sup>, T.Y. Mehta<sup>2</sup>, J.V. Solanki<sup>3</sup>, U.C. Rao<sup>1</sup>.* 1) GeneHealth, Green Cross Blood Bank & Genetics centre, Paldi, Ahmedabad; 2) Samarpan Medical & Research Organization, Modasa; 3) Department of Animal Genetics & Breeding, Veterinary college, Gujarat Agriculture University, Anand, India.

Hidradenitis suppurativa (HS) (OMIM 142690) is considered a chronic disease of apocrine glands. It usually develops in the groin and sometimes under the arms and under the breasts. The risk of developing any cancer with patients with HS is high as compared with others (*Arch Dermatol* 137:730-73, 2001). Several small and moderate families with autosomal dominant mode of inheritance have been reported (*Br J Dermatol* 142(5): 947-53, 2000), however the genes responsible for HS have yet to be identified. We have studied a large four generation Indian pedigree with autosomal dominant HS. The pedigree consists of 68 individuals including twenty affecteds (12 males/8 females). The age of onset was during puberty and the phenotype appeared to be 100% penetrant in this family. The expression of the phenotype was variable and ranged from very severe to moderate with typical features of HS. Detailed pathologic examinations were performed including histopathological studies in selected affecteds. The majority of the examined individuals were severely affected and their findings included cutaneous scars, folliculitis, GI polyps, familial gall stones, sinuses axillae, polymorph function defects, pilosebaceous abscesses and folliculitis. Hirsutism was observed in affected females. Skin grafting was performed in some of these individuals and two affecteds died due to squamous cell carcinoma. To our knowledge, this may be the biggest family with several affecteds. Blood DNA samples have been collected from selected individuals for future research. [u\\_c\\_rao@hotmail.com](mailto:u_c_rao@hotmail.com).

**The Azorean population: A genetic resource for the study of Congenital Heart Defects.** *R. Anjos<sup>1</sup>, T. Cymbron<sup>2</sup>, C. Macedo<sup>3</sup>, C.P. Duarte<sup>3</sup>, L. Mota-Vieira<sup>2</sup>.* 1) Pediatric Cardiology Dept., Hosp. Santa Cruz, Lisbon,; 2) Genetics & Molec Pathol Unit,; 3) Pediatric Dept. Hosp. Divino Espirito Santo (HDES), Azores Islands, Portugal.

Congenital heart defects (CHD) are the clinical manifestation of anomalies in embryonic cardiac development and constitute the most common form of clinically significant birth defects. This project aims to study CHD in the Azorean population. Located in the Atlantic Ocean, the Azores was first settled during the 15th century mainly by native Portuguese. The current population of 241,763 inhabitants grew over 22 generations almost entirely through reproduction, as immigration subsequent to founding was minimal. Azoreans live mostly in small rural localities, showing great similarity in life style and eating habits. Recently, we showed that this population is relatively homogeneous and has the highest value of consanguinity in Portugal. This project is based on the Azorean Register of CHD, established in 1992 at the HDES, the central Hospital of the archipelago. Our strategy includes a database with clinical data and detailed pedigree information, and a biobank of DNA, RNA, serum and frozen cells. Special attention will be taken in the reconstruction of extended multigenerational pedigrees, which greatly improves the statistical power of genetic analysis. This resource is a valuable tool to investigate the epidemiology of CHD, to characterize mutations present in candidate genes associated with CHD sub-phenotypes and to assess the effect of consanguinity in the CHD families. To date, 383 patients were clinically evaluated and classified according to the predominant cardiac lesion. Cardiac malformations affect 11 per 1,000 live births in Azores, a frequency 40% higher than in the general population. We are currently analysing the geographic distribution of CHD per island, the pedigree size and the family structure. With the exception of Corvo, all islands present CHD cases. The highest frequency was found in Santa Maria island and the lowest in Flores island. Moreover, we have evidence for familial aggregation in some rural localities, mainly in S. Miguel island. Funded by DRCT. (tcymbron@hdes.pt).

**A Rare Cause of Congestive Heart Failure in an Infant: First Reported Case of Infantile Marfan Syndrome in the Philippines.** *D.D. Cheng<sup>2</sup>, E. Cutiongco<sup>1</sup>, J. del Rosario<sup>2</sup>.* 1) Institute of Human Genetics, National Institutes of Health, University of the Philippines Manila; 2) Section of Cardiology, Department of Pediatrics, College of Medicine, University of the Philippines Manila.

A 2-month old boy presented with difficulty of breathing, irritability and feeding intolerance. Physical examination showed a poorly nourished infant in severe cardiorespiratory distress with associated tachycardia, poor pulses, holosystolic murmur at the apex and gallop. Extracardiac findings included hepatomegaly, pectus excavatum, arachnodactyly and dolichostenomelia. Chest radiograph revealed cardiomegaly with left pleural effusion. Electrocardiogram results showed sinus rhythm with normal axis for age with right and left atrial, and right ventricular hypertrophy. Echocardiography showed tricuspid and mitral valve prolapse with severe regurgitation, aortic root dilation and biatrial enlargement. Anti-congestive heart failure treatment was instituted with some improvement. Genetic and ophthalmologic consultations confirmed the clinical diagnosis of Marfan syndrome. Cardiac manifestations of Infantile Marfan syndrome in contrast to Classic Marfan syndrome are discussed.

**Chromosomal 22q11 deletion in conotruncal heart defects.** *YS. Choy<sup>1</sup>, HA. Latiff<sup>2</sup>, SK. Tan<sup>3</sup>, M. Alwi<sup>2</sup>, H. Samion<sup>2</sup>, G. Kandhavello<sup>2</sup>, LH. Ngu<sup>1</sup>, Azizion<sup>3</sup>.* 1) Genetics Unit, Pediatric Institute, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia; 2) National Heart Institute, Kuala Lumpur, Malaysia; 3) Cytogenetic Unit, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia.

Conotruncal heart defects are the commonest cardiac lesions found in chromosomal 22q11 deletion with significant implications in cardiac management beside overall management of the patient. A prospective randomized study was designed to study 22q11 deletion in patients with conotruncal heart defects. A total of 197 patients with conotruncal heart defects were tested for 22q11 deletion by FISH from July 2002 to Mac 2003. Patients with a known syndrome were excluded. The primary diagnosis were Tetralogy of Fallot (n=84), pulmonary atresia with VSD (n=52), truncus arteriosus (n=14), interrupted aortic arch (n=14), VSD with posterior malalignment of ventricular septum (n=7), simple TGA (n=12), and complex TGA (n=14). Dysmorphic features characteristic of 22q11 deletion were found in 33 patients (16.7%) and 27 patients (13.7%) had hypocalcemia. 22q11 deletion was found in 29 patients (14.7%). The deletion was positive in 28.8% of patients with PAVSD, 28.6% of patients with truncus arteriosus and 28% of interrupted aortic arch (75% of interrupted aortic arch type B) and 5.9% of patients with Tetralogy of Fallot. None of the patient with TGA or VSD and posterior malaligned ventricular septum was 22q11 deleted. Univariate analysis showed PAVSD, truncus arteriosus, interrupted aortic arch (particularly type B), right sided aortic arch, the presence of multiple aortopulmonary collaterals, hypocalcemia, presence of characteristic dysmorphic features to be significant predictors for 22q11 deletions. On multivariate analysis however, only PAVSD, truncus arteriosus, interrupted aortic arch and hypocalcemia were significant predictors for the deletion. In conclusion, testing for 22q11 deletion should be carried out for patients with isolated PAVSD or truncus arteriosus or interrupted aortic arch or any patients with conotruncal heart defects and hypocalcemia. The test should only be done in other patients with conotruncal heart defects after a careful clinical genetic evaluation.

**Abnormal mitochondria in arrhythmogenic right ventricle disease (ARVD).** *C. Friedrich, C. Gaymes.* Div Med Gen and Pediatric Cardiology, U Mississippi Medical Ctr, Jackson, MS.

A 15-year-old girl in excellent health developed a headache with facial flushing and died while bathing. She had no history of cardiac or neurologic symptoms. Her autopsy revealed an enlarged, markedly dilated, flabby, thin-walled right ventricle. The free wall was thin with adipose tissue extending to the endocardium with patchy fibrosis, with a 3.5 cm white-tan patch. There was pulmonary and generalized visceral congestion with herniation of unci and the cerebellar tonsils. Her psoas muscle showed Type 1 predominance, lipid deposition, scattered ragged red myofibers, and decreased cytochrome oxidase staining. On EM the mitochondria were large and exhibited rigid needle-like paracrystalline structures. Cristae were irregular and overlapping and some showed focally rounded distensions. Many had rounded osmiophilic condensations. She had hepatomegaly with marked microsteatosis. On EM her hepatocytes showed lipid and lipofuscin deposition, numerous large mitochondria, some expanded with flocculent material, some with swollen cristae, some with rounded osmiophilic condensations, and some mitochondria had fine cristae that overlap each other. Her father died at 53 of an MI. There were no other premature deaths. Her 17-year-old brother is in excellent health, with normal cardiac and neurological examinations. His skin, hair and nails were normal. His EKG, echocardiogram and exercise stress test were normal. His CPK was 4772 u/L (normal: 24-195). Aldolase was 43.2 u/L (normal: <8.3). His lactate, pyruvate, and the L/P ratio were normal. His ESR was zero. His urine organic acids, plasma carnitine levels and acylcarnitine profile were normal. A cardiac MRI is pending. Seven loci for familial ARVD have been mapped and two genes (*RYR2* and *DSP*) identified. Elevations of CPK and aldolase are not typical in ARVD. Mitochondrial abnormalities have not been reported. How myocardium is replaced by adipose tissue and fibrosis is unclear, although the arrhythmias may be due to disturbances of calcium homeostasis. The novel mitochondrial abnormalities seen on autopsy in this case may be a clue to the pathophysiology seen in this group of disorders.

**Autonomic Dysregulation in 22q Deletion Syndrome.** *O. Caluseriu<sup>1, 2</sup>, T. Tayyeb<sup>1</sup>, E.W.C. Chow<sup>1, 3</sup>, A.S. Bassett<sup>1, 3</sup>.*  
1) Centre for Addiction & Mental Health, Toronto, ON, Canada; 2) Hospital for Sick Children; 3) University of Toronto.

22q Deletion Syndrome (22qDS) is a microdeletion syndrome, commonly presenting with learning disabilities and congenital heart defects (CHD). Mortality in infants with 22qDS is often associated with major CHD but a recent study suggested that abnormal autonomic regulation of the vascular system may sometimes be responsible (Shashi et al, 2003). We comprehensively reviewed lifetime medical records for 72 adults with 22qDS for evidence of autonomic abnormalities such as severe hypotension or body temperature dysregulation. Three subjects had a history of unexplained hypotension that did not respond to sympathomimetics (inotropes). Subject 1 had tetralogy of Fallot (TOF) and on three separate occasions (at 2 months of age and twice at 2.5 years) presented an unexplained low cardiac output which responded poorly to inotropic agents. One of these events was accompanied by unexplained hyperthermia to 40.5 C with no identifiable focus of infection and negative blood cultures. Subject 2 during a second corrective surgery for TOF at age 6 years had a crisis on induction of anaesthesia with severe persistent hypotension (mean blood pressure of 20 mm Hg systolic) and tachycardia that did not respond to inotropes. Subject 3 had renal agenesis, recurrent hypocalcemia with aberrant subclavian artery. At age 18 years she developed pancreatitis after elective ERCP procedure; no signs of infection or necrosis of pancreas on CT were found. She developed persistent fever (38.9 C) but 48-hour cultures were negative. She became acutely unstable with blood pressure falling to a mean of 50 mm Hg. Increasing doses of inotropes were started but a second episode of refractory hypotension lead to cardiac arrest. Our data indicate it is possible (subjects 1 and 2) to survive autonomic system regulatory abnormalities in 22qDS but that death may occur (subject 3) even in the absence of major CHD. These data support previous case reports of 22qDS patients with abnormal autonomic regulation. This is a rare but important feature that can have dramatic consequences for 22qDS patients in view of their frequent need for invasive procedures and surgeries.

**Case report: jaundice due to biliary atresia with cardiac malformation and, umbilical and inguinal hernia. L.**

*Cornejo*<sup>1,2</sup>, *A. Molina*<sup>1</sup>, *I. Luna*<sup>1</sup>, *A. Noriega*<sup>1</sup>, *D. Harappe*<sup>1</sup>. 1) Genetics, Hospital del Niño DIF, Pachuca de Soto, Hidalgo, Mexico; 2) Area Academica de Medicina, Instituto de Ciencias de la Salud, Universidad Autonoma del Estado de Hidalgo.

**INTRODUCTION:** Biliary atresia and cardiac anomaly could be manifested as isolated malformations, or, they have been present as part of a syndromic disease. Differential diagnostic should be done with: biliary atresia, extrahepatic (OMIM 210500), Cat Eye Syndrome (OMIM 157450), Kabuki Syndrome (OMIM 147920), and, Alagille syndrome (OMIM 118450). **OBJETIVE:** To describe a patient with biliary atresia, extrahepatic, cardiac malformation and, umbilical and inguinal hernia. **CLINICAL DATA:** Male patient who was 5 months old when he was attended at the Hospital del Nio due to jaundice and fever. He was the first product gestation from a mother who was 27ys old and a father being 30ys old at conception time. Apparently, the gestation process was normal, spontaneous abortus and teratogenic data were negative. Delivery was done through cesarean section after forty weeks of gestation. Consanguinity was negative. His mother died when he was 3 months old. After clinical evaluation, he showed: microcephaly, none facial dismorphism; neck and thorax were normal; umbilical hernia; lefted inguinal hernia, male genitals; both, superior and lower extremities were normal. He also showed: severe mental retardation; ophthalmologically normal; atrial septal defect; normal hepatic function; biliary atresia, extrahepatic from hepatic biopsy; cervical and lumbar x-ray were normal; cerebral CT-Scan with cortical-subcortical atrophy. **DISCUSSION:** Infectologic test was not done. It was not recorded data for Cat Eye Syndrome, or, Kabuki Syndrome. It was thought in biliary atresia, extrahepatic, and, Alagille syndrome as differential diagnostics, last one was negative also. **CONCLUSION:** The present case report represent an biliary atresia, extrahepatic associated to cardiac malformation, and, hernial anomalies, last ones not previously reported. Counseling will be given as multifactorial disease.

**Phenotype of a 66 year old man with the 22q11 deletion syndrome.** *T. Ohki*<sup>2</sup>, *M. Wolfson*<sup>3</sup>, *A. Shanske*<sup>1</sup>. 1) Center for Craniofacial Disorders, Children's Hospital at Montefiore, and; 2) Vascular and Endovascular Surgery, Department of Surgery, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; 3) Institute for Basic Research, George A. Jervis Clinic, Staten Island, New York.

The chromosome 22q11 deletion syndrome includes the velocardiofacial syndrome (Shprintzen), Di George syndrome and conotruncal anomaly face syndrome. The prevalence is estimated to be 1/4500. The phenotype is highly variable and includes at least 150 associated anomalies. Most phenotypic studies are of children ascertained as a result of craniofacial or cardiac anomalies. We describe the phenotypic and, in particular, the vascular findings in a 66 year old man. S.O. is a 66 year old man referred by psychiatry with the deletion 22q11 syndrome. A cleft palate was repaired as a child and he now has low-frequency sensorineural hearing loss and bilateral cataracts. There is no cardiac history. He has unilateral renal agenesis and recently had surgery for prostatic hypertrophy. He had a seizure disorder many years ago and is being treated for hypothyroidism and hypoparathyroidism and has thrombocytopenia without giant platelets or a bleeding diathesis. He has a history of chronic candidiasis and basal ganglia calcifications. He has resided in a group home since his teenage years because of mental illness and retardation. His first psychiatric hospitalization was at 16 years. His psychiatric diagnosis is schizoaffective disorder and moderate mental retardation. His physical examination revealed a short well-nourished bald adult male with a blunt affect and euthymic mood and unclear speech. The pinna were normal, the palate was intact except for a bifid uvula, and the nasal tip was bulbous. Bilateral carotid bruits were audible and he had extensive varicosities of both lower extremities. Additional studies of adults with the 22q11 deletion syndrome are necessary to delineate the phenotype in adults and to better understand the natural history of the syndrome. The most comprehensive review of the subject to date (Cohen, 1999) reported on only a single adult over 50 years of age. Our patient has fewer major anomalies and different vascular findings than patients ascertained through a pediatric clinic.

**Arrhythmogenic right ventricular cardiomyopathy linked to 3p25: ventricular dysrhythmias and early sudden death, prevented in males with implantable cardioverter defibrillator therapy.** *K. Hodgkinson<sup>1</sup>, P. Parfrey<sup>1</sup>, S. Stuckless<sup>1</sup>, L. Thierfelder<sup>2</sup>, E. Dicks<sup>1</sup>, A. Bassett<sup>3</sup>, S. Connors<sup>4</sup>.* 1) Patient Research Centre, Health Science Centre, St John's, NL Canada; 2) Max-Delbruck Centre, Berlin, Germany; 3) Clinical Genetics Research, University of Toronto, On, Canada; 4) Division of Cardiology, Health Science Centre, St. John's, NL Canada.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) in Newfoundland is an autosomal dominant disorder, linked to a founder haplotype at 3p25, with high morbidity and mortality. In 11 large well ascertained families 367 subjects at 50% risk were identified: 197 were at high-risk (HR; based on haplotype data, obligate carrier status or early sudden death), 91 at low-risk (LR; based on haplotype), and the remainder were unknown. 24-hour Holter monitoring was done to assess number of premature ventricular complexes (PVCs) in 136 subjects (91HR: 44M, 47F, and 45LR; 21M, 24F). Forty eight HR subjects (30M, median age 32y, 18F, median age 41y) received an implantable cardioverter defibrillator (ICD) and were compared to controls with no ICD (matched on sex, age, family and risk status). We hypothesized that increased numbers of PVCs may be diagnostic in ARVC. Using 1000 PVCs in 24 hours in the age group 25-39, 100% M (n=21) and 41% F (7/17) with a first Holter fell above the cut-off. No LR subject (21M, 24F) had more than 1000 PVCs. These figures remain the same if a cut-off of 200 PVCs in 24 hours is used. Median age of death is 41 y (95% CI 38-44y) for males and 71 y (95% CI 64 -79y) for females: a relative risk of death M: F of 4.8 (95% CI 2.9 8.1). To date, the 5-year male mortality rate following ICD is zero vs. 28% in controls (p=0.009). The same (non-significant) trends were observed for females. Males are 100% penetrant for multiple PVCs by age 40y, have a higher prevalence of multiple PVCs at a younger age, and a much higher risk of early sudden death than females, which can be prevented by ICD therapy.

**Inheritance analysis of congenital left ventricular outflow tract obstruction (LVOTO).** *K. McBride<sup>1</sup>, M. Lewin<sup>2</sup>, R. Pignatelli<sup>2</sup>, S.D. Fernbach<sup>1</sup>, P. Schliekelman<sup>3</sup>, A. Combes<sup>1</sup>, A. Menesses<sup>1</sup>, W. Lam<sup>1</sup>, L.I. Bezold<sup>2</sup>, N. Kaplan<sup>4</sup>, J.A. Towbin<sup>2</sup>, J.W. Belmont<sup>1</sup>.* 1) Mol and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Division of Cardiology, Department of Pediatrics, Baylor College of Medicine, Houston, TX; 3) Statistics Department, University of Georgia, Athens, Georgia; 4) Environmental Diseases & Medicine Program, Biostatistics Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

**INTRODUCTION:** Left ventricular outflow tract obstruction malformations (prevalence 8/10,000), are suspected to have a substantial genetic component. In a large echocardiography study, we demonstrated a relative risk for bicuspid aortic valve (BAV) of  $>5$  and a heritability of 0.50 for first-degree relatives of children with aortic valve stenosis (AVS), BAV, aortic coarctation (CoA), or hypoplastic left heart syndrome (HLHS). To further define the inheritance pattern, we performed formal segregation analysis on this echocardiography study group. **METHODS:** We sequentially ascertained 95 probands with AVS, BAV, CoA, or HLHS and their families, for a total of 331 individuals. The symptomatic probands and all asymptomatic relatives with BAV were collapsed into a binary trait. Segregation analysis was performed under S.A.G.E. version 4.3, which utilizes a regressive multivariate logistic model for binary traits. Five models of inheritance were constructed, consisting of recessive, codominant, dominant, dominant with reduced penetrance, and no major gene. Multiple relative risk (MRR) was used to estimate the number of loci. **RESULTS:** All of the Mendelian inheritance models were significantly different from the general model, and thus rejected. The MRR analysis indicated a high likelihood for a limited number of loci, highest for 1-2 loci with a decline of likelihood dropping below the support level at 10 loci. **CONCLUSION:** A specific Mendelian inheritance pattern could not be supported with our family data. MRR analysis showed a high likelihood for a small number of loci. This data suggests linkage analysis could be fruitful in identifying the causative gene or genes in LVOTO malformations.

**Noonan syndrome and cardiomyopathy: outcome indicators.** *B.A. Salbert<sup>1</sup>, A. Lowe<sup>2</sup>, D. Connuck<sup>1</sup>, J. Towbin<sup>3</sup>, S. Colan<sup>4</sup>, G. Cox<sup>5,6</sup>, L. Sleeper<sup>2</sup>, E. Cadregari<sup>1</sup>, S. Clunie<sup>3</sup>, J. Messere<sup>6</sup>, S. Lipshultz<sup>1</sup>.* 1) University of Rochester and The Golisano Children's Hospital at Strong, Rochester, NY; 2) New England Research Institutes, Watertown, MA; 3) Baylor College of Medicine, Texas Children's Hospital, Houston, TX; 4) Harvard Medical School, Children's Hospital of Boston, Boston, MA; 5) Genzyme Corporation, Cambridge, MA; 6) Children's Hospital of Boston, Boston, MA.

In an effort to provide counseling for families, we reviewed long term data on patients with Noonan syndrome(NS) and cardiomyopathy(CM) focusing on the association between survival and age at diagnosis, congestive heart failure (CHF) at CM diagnosis, gender, and race/ethnicity. The Pediatric Cardiomyopathy Registry (PCMR), sponsored by a grant from the NHLBI, has 62 patients with NS diagnosed with CM between 1990 and 2002. All had hypertrophic CM. Annual followup data was collected from the time of diagnosis until death, transplant, transfer to a non-participating institution or discharge from followup. Of the 62 cases, 39% were retrospective(diagnosed 1990-1995) and 61% were prospective(1996-2002). Forty-seven(76%)had an etiology of NS at the time of diagnosis. Half of the NS patients were diagnosed with CM at <6 mo. of age, 27% had CM at the time of diagnosis, and 52% were male. The racial/ethnic breakdown was 64% white, 11% black, 20% hispanic, 5% other. The median followup time for these patients was 3.7 yrs. with 15 deceased(24%). Of the deceased, 93% were diagnosed with CM at <6 mo. of age as compared to 36% of those alive. One-year survival since the diagnosis of CM was lower in those diagnosed at <6 mo. of age than those diagnosed at >6 mo. of age(59% vs. 95% respectively, log-rank p-value<0.001). If CHF was present at the time of diagnosis of CM, one-year survival was lower than if CHF was not present(39% vs. 87%, log-rank p-value<0.001). One-year survival was similar between males and females(74% vs. 76%, log-rank p-value=0.767). One-year survival also did not differ by race(white 80%, Black 71%, Hispanic=50%, other=100%, log-rank p-value=0.181). Age of <6 mo. and CHF at the time of diagnosis are associated with poor outcome in patients with NS and CM.

**The phenotypic expression of poor-R wave progression on ECG in arrhythmogenic right ventricular**

**cardiomyopathy linked to 3p25.** *S. Stuckless<sup>1</sup>, K. Hodgkinson<sup>1</sup>, M. Norman<sup>2</sup>, A. Healey<sup>3</sup>, A.M. Whalen<sup>3</sup>, L. Thierfelder<sup>4</sup>, E. Dicks<sup>1</sup>, A. Bassett<sup>5</sup>, P. Parfrey<sup>1</sup>, S. Connors<sup>3</sup>.* 1) Patient Res Ctr, Health Sciences Ctr, St Johns, NL, Canada; 2) Finley Park Hospital, London, England; 3) Division of Cardiology, Health Sciences Ctr, St Johns, NL, Canada; 4) Max-Delbruck Centre, Berlin, Germany; 5) Clinical Genetics Research, University of Toronto, Ontario, Canada.

Eleven families with autosomal dominant arrhythmogenic right ventricular cardiomyopathy (ARVC) linked to a founder haplotype at 3p25 are ascertained in Newfoundland. Five additional families cannot be defined by DNA haplotype analysis due to the death of all affected individuals. Clinical diagnosis for ARVC in the absence of a genetic test is important. We examined the diagnostic utility of poor R-wave progression (PRWP:in the absence of septal myocardial infarction) in leads V1-V3 in 191 well-ascertained subjects from the 11 families. Risk status was determined as high (HR; n=110; 54M, 56F) based on haplotype data, obligate carrier status or early sudden death, or low (LR; n=81; 40M, 41F) based on haplotype. Of 43M under 40y with a first ECG, 47% had PRWP vs. 80% (8/10) over 40y. No LR M (n=31) under 40y had PRWP vs. 1/8 (12.5%) over 40y. Of 36 F under 40y with a first ECG, 11/36 (30%) had PRWP vs. 17/20 (85%) over 40y. Of 29 LR female under 40y, 1 (3%) had PRWP vs. no female over 40y. Specificity and positive predictive value are 100% for males in the age group 25-54y. Of 29 HR males who did not have PRWP on first ECG, 7 went on to develop this feature and 15/28 HR females developed PRWP. The median age of onset is 46y for both males and females. The development of PRWP on ECG is age dependant but not gender dependant. When account is taken of age, PRWP is a good diagnostic, inexpensive, accessible test for ARVC in these families, when genotyping is not possible.

**PHENOTIPICAL GENETIC VARIABILITY OF ECTODERMIC DYSPLASIA IN A PEDIATRIC HOSPITAL.**

**STUDY OF FIVE DIFFERENT MEXICAN FAMILIES.** *C.N. Gil<sup>1,7</sup>, J.M.R. Aparicio<sup>2,7</sup>, M.P. Barrientos<sup>3</sup>, W.B. San Martin<sup>4</sup>, M.V.C. Mendez<sup>2</sup>, L.H. Hurtado<sup>5</sup>, E.R. Balbuena<sup>6</sup>, L.A.S. Gonzalez<sup>7</sup>, R.T. Tenorio<sup>7</sup>, F.C. Salinas<sup>8</sup>.* 1) Estomatology; 2) Genetics; 3) Endocrinology; 4) Maxilofacial Surgery; 5) Cytogenetics; 6) Dermatology, Hospital para el Nino Poblano, Puebla, Mexico; 7) Estomatology, Benemerita Universidad de Puebla, Mexico; 8) Oral genetics, Medical University South Carolina, USAg.

**INTRODUCTION:** Ectodermic dysplasias form a heterogenic group characterized by the involvement of embryo-ectodermic tissue: teeth, nails, ophthalmologic signs, labial-palatinum grooves sweat glands, and hair. **CLINICAL CASES:** Although all 5 syndromes in this study had different grade of phenotypic variability, the most common clinical characteristics were: anhidrotic ectodermic dysplasia (AED), the most frequent form and characterized by hypo- or anodontia, unguis dysplasia, varying alopecia, more or less severe dysmorphia (forehead bumps, rings under the eyes, etc.), and notably a hypo- or anhidrosis, including one patient without saliva production. All patients showed discrete hyperpigmented dermatological lesions, variability in alopecia manifestations. In one case the father was also diagnosed as ectodermic dysplasia with few saliva production. One case was diagnosed as cardiofacio cutaneous syndrome that has no dental alterations. **GENETICS:** For this same phenotype, several transmission modes have been published: recessive related to the X chromosome, and less frequently autosomal dominant or recessive. Two genes have been identified before: EDA1 for the X-related form and EDA3 localized on the 2q13 chromosome. EDA3 is implied in the AED transmitted in the autosomal dominant and recessive forms. A fourth gene incriminated in autosomal recessive AED has recently been identified. The gene, EDARADD, is located on chromosome 1. The protein it encodes is associated with the Edar intracellular domain and consequently in the Ectodysplasin-Edar. **CONCLUSIONS:** It is important for special third level diseased patients with congenital or genetic disorders to work in a multidisciplinary way in order to offer the best rehabilitation therapy for a better quality of life.

**Hyperactivity in individuals with Autistic Disorder.** *R.K. Abramson<sup>1</sup>, A.V. Hall<sup>1</sup>, S.A. Ravan<sup>1</sup>, M.L. Cuccaro<sup>2</sup>, C.M. Wolpert<sup>2</sup>, M.A. Pericak-Vance<sup>2</sup>, H.H. Wright<sup>1</sup>.* 1) Dept Neuropsychiatry & Behav, Wm S Hall Psychiatric Inst, Columbia, SC; 2) Duke University Center for Human Genetics, Durham, NC.

Hyperactivity is reported in a subset of probands with Autistic Disorder (AD). AD individuals are often characterized by their level of adaptive functioning. This study compares AD males and females with high adaptive functioning (AD-H) to those with low adaptive functioning (AD-L) to see if hyperactivity will discriminate between the two groups. Subjects (82 male and 30 female) came from a pool of 150 AD participants [125 Caucasian (C) and 25 African-American (AA)] in the Duke/USC collaborative study. Diagnoses were confirmed by the Autism Diagnostic Interview-Revised (ADI-R) (Lord, 1995). Hyperactivity was measured by the Aberrant Behavior Checklist Community subscale for hyperactivity (ABC-H) (Aman, 1985) and by the SNAP-IV (Swanson, 2001). ABC-H scores were not significantly different for AA versus C probands ( $F=0.013$ ,  $p=0.987$ ,  $df=2$ ). Using Vineland Adaptive Behavior Scale supplemental norms (Carter, 1998), AD-H was defined as 60th percentile or greater and AD-L as below the 60th percentile. Males and females were represented differentially in the high and low groups (Chi square = 3.69, z score = 1.92, which exceeded the critical value of  $z = 1.67$ ). ABC-H scores did not differentiate the 44 AD-L probands from the 68 AD-H probands. A significant gender by group interaction effect showed that AD-L males had higher ABC-H scores than any other group ( $F=5.99$ ,  $p=0.016$ ,  $df=1$ ). AD-L males had higher SNAP IV scores than AD-H males ( $t=2.137$ ,  $p=0.038$ ,  $df=48$ ). AD-L females had the lowest ABC-H scores of any group but there was no difference between AD-L and AD-H female ABC-H scores. This study supports previous findings that females as a group had lower functioning than males. However, ADHD symptoms may discriminate low and high functioning males with autism. This pattern did not hold true for the female participants.

**A novel mutation in the Caveolin-3 gene causing familial isolated hyperckemia.** *P. Gallano<sup>1</sup>, L. Alías<sup>1</sup>, I. Ferrer<sup>2</sup>, A. Freixas<sup>1</sup>, M.J. Rodríguez<sup>1</sup>, M. Baiget<sup>1</sup>, M. Olivé<sup>2</sup>.* 1) Dept Genetics, Hosp Sant Pau, Barcelona, Spain; 2) Dept Neuropathology, Hosp Bellvitge, Barcelona, Spain.

Caveolin-3 is the skeletal and cardiac muscle-specific protein of the caveolin gene family and the major component of the caveolae complex. Mutations in the CAV-3 gene cause Limb girdle muscular dystrophy type 1C (LGMD1C), Rippling muscle disease (RMD), Distal myopathy and Hyperckemia. We report a family suffering from isolated hyperckemia originated by a novel mutation in the CAV-3 gene. The evaluation of the patient (22-year-old, good sportsman and without muscular weakness) showed mild bilateral calf hypertrophy, normal EMG and elevated serum CK levels. His mother presented identical features. Caveolin-3 was reduced in the muscle biopsy analyzed by immunohistochemical and Western blot studies. Dysferlin was reduced in immunohistochemical analysis but normal in Western blot. A C->A transition in 169 nucleotide position, causing Valine to Methionine change in 57 codon of the polypeptide, was detected in exon 2 of CAV-3 gene. This change was confirmed as a novel missense mutation after the analysis of 50 normal individuals, 10 calpainopathies, 10 sarcoglycanopathies, 5 dysferlinopathies, 15 dystrophinopathies and 10 spinal muscular atrophies. This V57M mutation is the second one described in the CAV-3 gene associated to familial hyperckemia. The fact of the existence of clinical heterogeneity associated to CAV-3 gene mutations could be due to the specific altered protein domain. Nevertheless, there are a few mutations described in that gene to conclude about phenotype-genotype correlations. We consider suitable the study of CAV-3 gene in isolated hyperckemia cases in whose dystrophin involvement was excluded.

**Neurofibromatosis type 1 (NF1): phenotypical variability in patients with scoliosis or pseudoarthrosis of the tibia.**

*E. Bonioli<sup>1</sup>, M. Russo<sup>1</sup>, G. Morcaldi<sup>1</sup>, C. Bellini<sup>1</sup>, M. Baldi<sup>1</sup>, S. Boero<sup>2</sup>, G. Stella<sup>2</sup>.* 1) Paediatric Department State University of Genova, Italy; 2) Orthopaedic Unit G. Gaslini Institute Genova, Italy.

About 30-50% of children with NF1 have orthopaedic complications. Among these, scoliosis is the most common, while pseudoarthrosis of the tibia is the principal lower leg deformity. Typical non orthopaedic complications (NOCs) of NF1 are: optic pathway gliomas, plexiform neurofibromas (PNF), epilepsy, hydrocephalus, Noonan phenotype, other CNS malformations (myelomeningocele, tumor brain, arachnoids cyst, Chiari malformation) and malignancy.

**PATIENTS:** We considered 184 children (73 M / 111 F; median age at diagnosis 7.9) with NF1. They were subdivided in three groups: A Group: 140 pts (63 M / 77F) without orthopaedic complications; B Group: 27 pts (10 M / 17 F) with severe dystrophic scoliosis; C Group: 17 pts (6 M / 11 F) with tibial pseudoarthrosis. NOCs were evaluated in all 184 pts. The three groups were therefore compared in order to evaluate any different frequency of NOCs. **RESULTS:** NOCs occurred in 73 group A pts (52.1%): PNF (19.3%); glioma (13.6%); epilepsy (9.3%); hydrocephalus (6.4%), other CNS malformations (5%), Noonan phenotype (5%), malignancy (0.7%). Frequency of NOCs in group B was 51.8%: PNF (33.3%); glioma (22.2%), other CNS malformations (22.2%); tumours extra-CNS (18.5%), epilepsy (7.4%), hydrocephalus (3.7%). Frequency of NOCs in group C was 17.6%; glioma (11.8%) and PNF (5.8%). The association of three or more NOCs in the same patient was observed in 3.6% pts of group A and in 38.5% of group B. No patient of group C had more than one complication. **DISCUSSION:** Incidence of NOCs in C group was significantly lower than in two other groups, while incidence of NOCs in group B was the same as group A. Moreover in C group there was no more than one NOC in the same patient, while in B group we observed more frequently the association of three or more NOCs in the same patient. Our data point to a lower incidence of NOCs in NF1 patients with pseudoarthrosis of the tibia.

**Assessment of the translocation frequency between 4q and 10q in the South African FSHD population: a pilot study.** *M. Alessandrini*<sup>1</sup>, *A. Van der Merwe*<sup>1</sup>, *C-M. Schutte*<sup>2</sup>, *A. Olckers*<sup>1,3</sup>. 1) Centre for Genome Research, Potchefstroom University, Pretoria, South Africa; 2) Dept of Neurology, University of Pretoria, South Africa; 3) DNAbiotec Pty (Ltd), Pretoria, South Africa.

Facioscapulohumeral muscular dystrophy (FSHD) is a progressive autosomal dominant myopathy, preferentially affecting facial, shoulder girdle and upper-arm muscles. It is one of the most common muscular dystrophies with a prevalence of 1 in 20,000. The rearrangement that results in FSHD entails a deletion of an integral number of 3.3 kb repeat arrays on chromosome 4q35. A candidate gene for this disorder has, however, yet to be identified. Data suggests a position effect variegation model resulting in FSHD. Southern blot analysis has led to the identification of an additional locus with more than 98% homology to 4q35, which was subsequently mapped to chromosome 10q26. This region may also display 3.3 kb repeat deletions, similar to chromosome 4q35. A deletion fragment located on chromosome 10q is, however, non pathogenic and does not result in FSHD, but rather compromises accurate molecular diagnoses. Previous data suggested a model for subtelomeric plasticity due to the occurrence of DNA translocations between the 4-type and 10-type arrays. The high degree of homology between these fragments may be the predisposing factor that has led to these translocations being observed in 20% of the Dutch population. The objectives of this study were to optimise the Bgl II-Bln I dosage test via a non-radioactive protocol and to verify the presence of translocation events between the 4q and 10q arrays of affected and unaffected individuals selected from South African FSHD families. Results obtained from this study suggest that 4q-10q plasticity is indeed observed in the South African FSHD population. However, the observed translocation frequency of 8.7% is considerably lower than that obtained from previously reported population studies. Since a relatively small cohort was investigated in this pilot study, additional analyses with a significantly larger sample size are foreseen.

**Synpolydactyly associated with an apparently balanced reciprocal 2;3 chromosome translocation.** *C. Moore<sup>1</sup>, M. Macera<sup>2</sup>, A. Babu<sup>2</sup>, A. Dagum<sup>3</sup>, L. Hurst<sup>3</sup>, L. Mehta<sup>1</sup>*. 1) Schneider Children's Hospital at North Shore, Manhasset, NY; 2) Wyckoff Heights Medical Center, Brooklyn, NY; 3) University Hospital, Stony Brook, NY.

A mother and daughter presented with surgically corrected, bilaterally symmetrical synpolydactyly of the hands and feet. Maternal family history was significant for variable synpolydactyly in a total of nine individuals over five generations. Digit anomalies in the child are best described as 4,5 syndactyly of the hands with a duplicated digit in the web, camptodactyly of digits 2,3, and incomplete metacarpal synostosis of digits 3,4; both feet had broad 1st digits, short 4th digits and mild 2,3,4 digit syndactyly. The mother was described as having 3,4 digit syndactyly of the hands, a duplicated digit in between and camptodactyly of digits 3 and 4. She had syndactyly of digits 4,5 with a 6th digit on both feet. Other family members are described to also have extra as well as fused digits with contractures. No other significant birth defects were detected in our patients. The 3 y.o. child had a history of hypotonia but was showing good developmental progress. Chromosome analysis revealed an apparently balanced reciprocal translocation  $t(2;3)(q32.2;q25.1)$  in the mother and the child. These findings indicate that the autosomal dominant synpolydactyly segregated with the chromosome translocation in these two individuals tested. It is of interest that the breakpoint on chromosome 2 is in the region of the HOXD gene cluster at 2q31-q32. Synpolydactyly (syndactyly type II) and other limb malformations are associated with mutations (such as amino acid substitutions and truncations), polyalanine expansions, and deletions of the HOXD13 gene located within this cluster. It is highly likely that the breakpoint on chromosome 2, in this inherited translocation, is located within the HOXD13 gene or adjacent to regulatory elements affecting expression of HOXD13. DNA analysis of the breakpoint and additional cytogenetic studies on affected family members will help determine if this is the first reported case of an inherited chromosome translocation causing synpolydactyly.

**A new Kabuki-like syndrome in siblings.** *B. Hall.* Pediatrics, Genetics, University of Kentucky, Lexington, KY.

Kabuki syndrome (Nikawa N, et al, Amer J Med Genet 31:565, 1988) is a distinct multiple congenital anomaly syndrome of unknown etiology. Most cases are sporadic occurrences in their families. The face is very distinctive with long appearing palpebral fissures, eversion of lower lateral eyelids, arched eyebrows, flat nasal bridge, and laterally protruding ears. I present male and female siblings with Kabuki-like face and multiple anomalies who represent an apparent phenocopy of the Kabuki syndrome.

Both pregnancies were uncomplicated except for Crohn's disease. During both pregnancies, mother took sulfasalazine the first trimester (Case 1) or Asacol (Case 1 and 2). Both children were IUGR, had poor postnatal growth, and were moderately psychomotor delayed. Each child's features included large laterally protruding ears, long appearing palpebral fissures, mild eversion of lower eyelids, long eyelashes, thick eyebrows, thin upper lip, inverted v-shape mouth, and small mandible. Case 1 (female) also had short incurved 5th fingers with small nails and microcephaly. Case 2 (male) had bilateral cryptorchidism and borderline macrocephaly. Both children lacked digital fat pads. Both had normal chromosome studies and CNS imaging studies.

While clinical differentiation appears adequate to separate out the above siblings from reported cases of Kabuki syndrome, identification of the etiology of Kabuki syndrome will greatly aid in excluding phenocopies or outliers.

**Is Autism Caused by Numerous Genetic and Cytogenetic Syndromes?** *S. Lucchese, N. Takahashi, J. Miles.* Dept Child Health, Med Gen Div, University of Missouri Hospital, Columbia, MO.

To learn whether chromosome abnormalities and genetic syndromes cause autism or merely lower the threshold in the genetically susceptible host, we examined etiologically distinctive groups of autism probands. The 3 study groups had either a cytogenetic abnormality (9), an autism related syndrome (6), or complex autism (defined on the basis of multiple dysmorphic features) (21). Each of the three study groups was compared with a group of 65 autism probands with essential autism (normal morphology, brain structure, HC>2%). Cytogenetic syndromes included: del(2)(q37.1), + ring 8, del(8)(p22.2), del(11)(q24), + der(15)t(4;15)(p16;q13), + isodic(15)(q13), + der(15)(q14), + (21), add(2)(q35), XXY. Syndromes were Tuberous Sclerosis, Fragile X, Sotos, Fetal Valproate and a private macrocephaly syndrome. All met DSM-IV diagnostic criteria. Results: Family histories of autism related disorders - alcoholism, affective disorders, OCD, seizures, cognitive and other psychiatric disorders were obtained. In all 4 groups, the family histories revealed comparably high levels of these neurologic and psychiatric disorders. The 3 study groups differed from the essential autism group in the sib recurrence risks and sex ratios, indicating genetic differences between them. The latter sib recurrence risk was higher when the proband had essential autism (9.2% (6/65)) compared to no risk (0/36) when the proband had a cytogenetic, genetic or complex autism ( $P=0.086$ ). Sex ratios were lower in the cytogenetic and complex groups, compared with essential autism (1.2:1 vs 7.8:1,  $p=0.013$ , 2.5:1 vs 7.8:1,  $p=0.08$ ). Family histories of psychiatric and cognitive disorders are assumed to indicate genetic loading that predisposes to the development of autism. For autism caused by a cytogenetic or genetic syndrome, one might assume that family loading would not be higher than in the general population. The similarity of family histories across the groups implies that for most cytogenetic and genetic syndromes, which occur in autism, their causal effect is only partial and may just lower the threshold for the development of autism.

**Intra-familial correlations of symptoms and clinical characteristics in multiplex Korean families with schizophrenia.** *K.S. Hong<sup>1</sup>, K-S. Choi<sup>1</sup>, S.W. Kim<sup>1</sup>, Y-S. Lee<sup>2</sup>, H.Y. Yoe<sup>3</sup>, E.Y. Cho<sup>1</sup>, Y.L. Jang<sup>4</sup>, W-S. Jang<sup>1</sup>, D.Y. Park<sup>1</sup>.* 1) Department of Psychiatry, Sugkyunkwan University School of Medicine, Samsung Seoul Hospital, Seoul, Korea; 2) Yong-In Mental Hospital, Yong-In, Kyunggi-Do, Korea; 3) Eumsung Mental Hospital, Eumsung, Korea; 4) National Chuncheon Hospital, Chuncheon, Korea.

This study aims at examining familial associations of symptoms and clinical characteristics in affected sibling or relative pairs of schizophrenia as an effort to identify genetically homogenous phenotypes in this complex genetic disorder. Seventy-five subjects with DSM-IV diagnosis of schizophrenia from 35 Korean families multiply affected with schizophrenia were ascertained from clinics of general hospitals and mental hospitals. Direct interviews were done using Korean version of Diagnostic Interview for Genetic Studies. Krawieka Rating Scale and The Schedule for the Deficit Syndrome were also applied for further evaluation of psychopathologies. Intra-familial concordances and correlations of clinical variables were tested using  $\chi^2$ -test and Spearman's correlation, respectively. Significantly high concordance rate within relative pairs was found for the diagnosis of paranoid vs. non-paranoid subtypes. Deficit vs. non-deficit syndromes showed only trend of concordance. Single item score of hallucination and factor score of negative symptoms showed significant intra-familial correlations. Ages at onset of prodromal symptoms, onset of recognized psychotic symptoms and first hospitalization did not show any correlation within the relative pairs. Familial factors, possibly the genetic factor contribute to the phenotypic characteristics of paranoid vs non-paranoid subtype, hallucination, and negative or deficit syndrome. It is suggested that these variables are candidates of phenotype marker for genetic linkage studies.

**Chromosomal Markers and their genotype/phenotype correlation- Preliminary data.** *L. McCarthy*<sup>1</sup>, *A. Maleki*<sup>2</sup>, *L. McGavran*<sup>1, 2</sup>, *C. Tsai*<sup>1</sup>. 1) Division of Genetics/Pediatric, The Children's Hospital, Denver, CO; 2) Colorado Cytogenetic Lab, Department of Pathology, The Children's Hospital, Denver, University of Colorado.

Supernumerary chromosomal markers are frequent findings during amniocentesis or routine chromosome testing. The incidence of supernumerary markers is approximately 1 in 2500. The genotype has been recognized for many years; however, insufficient information was available genetic counseling. These results from difficult characterization of the small markers by traditional cytogenetic technology and lacking long term follow up literature on the result of the pregnancy. We herein report the preliminary data on the study we conduct to correlate supernumerary marker genotypes to their specific phenotype. With the advance of molecular cytogenetic techniques, including florescence in-situ hybridization (FISH), these markers can be more clearly described. Sixty-five patients, including adults, children and markers identified during prenatal testing, CVS or amniocentesis, with supernumerary markers ascertained through the Colorado Cytogenetics Laboratory over the last ten years were invited to this study. We also ascertain the on-going new patients identified through the lab and the genetic clinic. Each patient who was enrolled in the study was asked to provide a complete history with medical records and undergo a physical exam. The endpoint of the study is to correlate a specific genotype with its phenotype. We summaries the preliminary data on seven patients participated to date for this conference. Complete data will follow. Hopefully this research will help us better understand the clinical affects of specific supernumerary markers and provide specific information for genetic counseling.

**Subtle unbalanced t(2;14)(p25;q32) uncovered by subtelomeric FISH in a patient with congenital anomalies.** *H.A. Overcash, S.J. Hassed, P.L. Wilson, J. Lee, S. Li, J.J. Mulvihill.* Department of Pediatrics, OUHSC, Oklahoma City, OK.

A six-month-old baby girl was referred for chromosomal evaluation because of multiple congenital anomalies. The dysmorphic features included: developmental delay, Dandy-Walker malformation, mild epicanthal fold of the right eye, wide nasal bridge, deep and short philtrum, slight widely spaced nipples, dimples on both elbows and knees and others. Routine chromosome analysis was performed and revealed that each of the cells had a derivative chromosome 2 with a small fragment of chromosomal material of unknown origin attached, plus a questionable inversion of chromosome 21q. None of parents were available for chromosome studies. To determine the origin of unknown chromosomal material, FISH was performed utilizing commercial probes corresponding to the subtelomeric regions of all 22 autosomes and the sex chromosomes, X and Y, except for the short arms of the acrocentric chromosomes 13, 14, 15, 21 and 22. All the cells analyzed had a der(2)(2;14)(p25;q32). Clearly, the minimal deletion of 2p25 and duplication of 14q32 contribute our patients clinical features, which will be compared to the previously reported limited number of cases with 2p- or 14q+.

**Revised diagnostic criteria for neurofibromatosis 2 (NF2).** *R.T. Ramsden*<sup>1</sup>, *D.G.R. Evans*<sup>2</sup>, *A.J. Wallace*<sup>2</sup>, *H. Joe*<sup>3</sup>, *M.E. Baser*<sup>4</sup>. 1) Department of Otolaryngology, Manchester Royal Infirmary, Manchester, UK; 2) Department of Medical Genetics, St. Mary's Hospital, Manchester, UK; 3) Department of Statistics, University of British Columbia, Vancouver, BC, Canada; 4) Los Angeles, CA, USA.

We recently reported that each of the four sets of clinical diagnostic criteria for NF2 had low sensitivity at the time of the initial clinical assessment, although the Manchester criteria were the most sensitive (Neurology 2002;59:1759-65). In this study, we propose and evaluate modifications to the Manchester criteria. The study had 163 NF2 patients in the United Kingdom registry who did not have a family history of NF2 and who presented without bilateral vestibular schwannomas; this group presents the greatest diagnostic difficulties. The proposed modifications are A) in people without a family history of NF2, permitting the diagnosis when there are multiple meningiomas and only one, instead of two, other tumors or cataract (as in the NNFF criteria), B) adding childhood mononeuropathy (ages  $\leq 10$  years) as a diagnostic criterion, and C) in addition to clinical criteria, permitting the diagnosis when constitutional *NF2* mutations are identified. We used Kaplan-Meier analysis to determine the time course, from initial assessment to the most recent clinical evaluation, of the increasing proportion of people who would be diagnosed with NF2 using the current Manchester criteria and the modifications. The jackknife method was used to compute pointwise standard errors for differences in proportions of pairwise Kaplan-Meier curves between different sets of criteria. Modification A significantly increased sensitivity above the Manchester criteria from 4-17 years after initial assessment. Modification B did not increase sensitivity above modification A, because all patients who were identified earlier by adding childhood mononeuropathy as a criterion had already been identified earlier using modification A. Modification C significantly increased sensitivity above modification A from initial assessment to 5 years after initial assessment. We conclude that simple modifications to the Manchester criteria significantly increase sensitivity from initial assessment to 17 years after initial assessment.

**Triploidy with a karyotype 69,XXX.** *G. P. Akpınar<sup>1</sup>, E. Ergül<sup>1</sup>, O. Karlı<sup>2</sup>, G. Turker<sup>2</sup>, M. Unlu<sup>3</sup>, K. Cetin<sup>1</sup>, A. Sazci<sup>1</sup>.*  
1) Department of Medical Biology and Genetics,; 2) Pediatrics,; 3) Radiology, Faculty of Medicine, University of Kocaeli, Derince, 41900, Turkey.

Triploidy is a chromosomal abnormality where three complete sets of the haploid genome are present, and occurs in about 2 percent of conceptuses. Most are lost as miscarriages, are responsible for about 20 percent of chromosomally abnormal spontaneous abortions. The live birth rate for triploidy is 1/50.000 live births. There seem to be three different mechanisms producing triploidy: firstly, diandry, secondly, likewise, digyny, thirdly and finally, dispermy. A triploid infant (karyotype 69,XXX), 1440 grams, 32 weeks gestation, 44 cm head circumference, was born with caesarean section. The mother was 22 years old and father 19 years old who used hashish with antisocial behavioural manifestations. Both had a gipsy origin. She had one abortion, a 2 years old healthy daughter, and a triploid daughter. The triploid baby had a problem with breathing and positioning the body, muscular hypotonia, aberrant skull shape, omphalocele, syndactyly of third and fourth fingers, short neck, dysplastic calvaria with large posterior fontanel, simian crease of the hands, pulmonary hypoplasia, low set ears. Left ear had an accessory in front of tragus. Eyes were spaced farther apart than usual. In MRI; agenesis of corpus callosum was observed. There were irregularities in posterior interhemispheric fissure and minimal interdigitation in cerebral sulcus. In abdominal USG; there were heart problems including VSD, and bilateral renal hypoplasia. In transfrontal USG; corpus callosum posterior was not formed. The baby did not have ability to suck. In addition, we also studied APOE, AT1, MTHFR polymorphisms in the four members of the family. They all had APOE3/3 genotype. Triploid baby and father had MTHFR A1298A genotype and mother and sister had MTHFR A1298C genotype. The family all had MTHFR C677C genotype. Father and triploid baby had AT1 AC genotype, mother and sister had AT1 AA genotype.

**Monosomy 1p36.** *C. Tsai.* Dept Pediatrics/Div Genetics, Childrens Hosp, Denver, CO.

Monosomy 1p36 is the most common terminal deletion syndrome. Most commonly associated features and medical problems in patients with this deletion syndrome include large anterior fontanelle, motor delay/hypotonia, moderate to severe mental retardation, growth delay, prominent forehead, microcephaly, deep set eyes, vision problems, seizures, flat nasal bridge, midface hypoplasia, clinodactyly and/or short fifth finger, low-set/ asymmetrical ear, hearing deficits behavioral problems and thyroid diseases. This contiguous gene deletion syndrome is presumably caused by haploinsufficiency of the involved genes. I am reporting 7 patients with chromosome 1p36 deletions ascertained through a tertiary childrens hospital pediatric genetic service. Their cytogenetic anomalies ranged from pure terminal deletions, interstitial deletions secondary to complex rearrangements, to subtelomere deletion. All are de novo cases. Other than one with 1p36.23 deletion and on has subtelomeric deletion distal to the commercial 1p36.3 FISH probe, the rest of the patients have 1p36.3 deletion. I summarized the findings according to the following perspective: hearing, palatal and ophthalmological, echocardiograms, ultrasound findings, neurological assessments (some with image studies), thyroid function tests, and developmental findings. The result demonstrated that subtelomeric deletion has relatively milder phenotype and not very characteristic in the facial features. One patient also demonstrated the importance of performing MTHFR mutation analysis because of the born haploinsufficiency and relatively common gene frequency of thermal labile mutation.

**A quantitative assessment of limb anomalies in CHARGE syndrome: Correlation with diagnosis and characteristic CHARGE anomalies.** *K.E. Brock<sup>1</sup>, M.A. Mathiason<sup>1</sup>, B.L. Rooney<sup>1</sup>, M.S. Williams<sup>2</sup>*. 1) Medical Foundation, Gundersen Lutheran Med Center, La Crosse, WI; 2) Dept. of Pediatrics Gundersen Lutheran Med Center, La Crosse, WI.

CHARGE syndrome was initially not thought to involve the limb. Several subsequent reports have shown that limb anomalies are not uncommon. To date, there have been no quantitative studies of limb anomalies in CHARGE syndrome. This study was designed to answer several questions: Do CHARGE patients with limb anomalies represent a subgroup within CHARGE syndrome? Are there correlations between certain CHARGE syndrome anomalies and limb anomalies? Are certain types of limb anomalies seen with increased frequency in CHARGE syndrome? **METHODS:** All described patients were categorized utilizing the expert diagnostic model proposed by Mitchell (1985). Patients with chromosomal anomalies or familial CHARGE were excluded, as were patients with inadequate clinical descriptions, and patients in large series where individual characteristics could not be ascertained. Multivariate analysis was performed. **RESULTS:** 172 patients with definite or probable CHARGE syndrome were analyzed. 37% of these patients have at least one limb anomaly. Limb anomalies were significantly associated with coloboma, urinary tract anomalies and genital anomalies when definite and probable CHARGE patients are analyzed together ( $p < 0.005$ ,  $p < 0.019$  and  $P < 0.026$  respectively). These associations were not significant when the definite or probable CHARGE patients were analyzed separately, with the exception of genital anomalies in definite CHARGE ( $p < 0.035$ ). Association with choanal atresia is significant only for probable CHARGE patients ( $p < 0.016$ ). **CONCLUSIONS:** Limb anomalies are present in more than one-third of CHARGE syndrome patients. Limb anomalies are seen more frequently in association with certain CHARGE anomalies, which could provide insight into common embryologic pathways. There is not a common limb anomaly seen in CHARGE syndrome, with the possible exception of dermatoglyphic abnormalities.

**NSD1 - deleted Sotos syndrome patients with delayed bone age. -Is the advanced bone age essential for Sotos syndrome?-. T. Doi<sup>1</sup>, T. Kondoh<sup>1</sup>, E. Kinoshita<sup>1</sup>, T. Matsumoto<sup>2</sup>, M. Hara<sup>1</sup>, S. Oka<sup>1</sup>, N. Kurotaki<sup>3</sup>, N. Harada<sup>3</sup>, N. Matsumoto<sup>3</sup>, N. Niikawa<sup>3</sup>, H. Moriuchi<sup>1</sup>.** 1) Dept Pediatrics, Nagasaki Univ Sch Medicine, Nagasaki, Japan; 2) Dept Nursing, Nagasaki Univ Sch Medicine, Nagasaki, Japan; 3) Dept Human Genetics, Nagasaki Univ Sch Medicine, Nagasaki, Japan.

Sotos syndrome is a malformation syndrome characterized by overgrowth from the prenatal stage through childhood, advanced bone age, macrocephaly, a distinctive facial appearance, and developmental delay. Recently, NSD1 was identified as a causative gene for Sotos syndrome and haploinsufficiency of the NSD1 is one of the main etiologies of Sotos syndrome. We here present two cases of NSD1 - deleted Sotos syndrome with delayed bone age. Patient 1 is now a five-year-old boy. At the age of 14 months, he was diagnosed as Sotos syndrome from such clinical features as tall stature of 79.4 cm (+1.9SD), heavy weight of 12.6 kg (+3.1SD), large head circumference of 51 cm (+3.4SD), frontal bossing, pointed chin, hypertelorism, down-slanting palpebral fissures, high-arched palate and developmental delay. NSD1 gene analysis revealed 2.2-Mb deletion in heterozygous state. At the age of 61 months, his height was 105.2 cm (+0.3SD), weight was 20 kg (+1.0SD), and bone age (52 months old) was 9 months behind. Brain MRI revealed cephalomegaly, ventriculomegaly, thinning of the corpus callosum and thinning of the pituitary fossa. Hormonal examinations showed normal secretion of growth hormones and thyroid function. Patient 2 is now a one-year-old girl. At 12 months of age her head circumference was 49 cm (+2.7SD), and height was 74 cm (mean). FISH using NSD1 as a probe showed only one signal in her peripheral lymphocytes, indicating heterozygous deletion of NSD1. CT scan of the brain revealed no abnormality. The carpal bones X-ray showed no appearance of capitate and hamate bone, indicating delayed bone age. The advanced bone age with overgrowth is one of major criteria of Sotos syndrome. However, our NSD1 - deleted Sotos syndrome patients showed delayed bone age that was not associated with hormonal abnormality, suggesting that the advanced bone age may be dispensable for Sotos syndrome and result from unknown factor(s) other than NSD1.

**MeCP2 mutations and gastrointestinal phenotype in Rett Syndrome.** *G. Bibat*<sup>1</sup>, *C. Cuffari*<sup>2</sup>, *S. Naidu*<sup>1</sup>. 1) Neurogenetics Unit Kennedy Krieger Inst, Baltimore, MD; 2) Division of Pediatric Gastroenterology, Johns Hopkins Hospital, Baltimore, MD.

Rett syndrome (RS), a neurodevelopmental disorder predominantly affecting females, results from mutations in the methyl-CpG-binding protein 2 (MeCP2) gene located on chromosome Xq28. Mutations in MeCP2 are associated with developmental regression and significant gastrointestinal (GI) dysfunction. The GI manifestations include: 1) constipation; 2) reflux; 3) dysphagia; 4) failure to thrive (z-score < -2SD), which vary in severity. To determine if the GI features may be genotype dependent, we evaluated 36 females with the phenotype of RS (ages 3-15 years with mean age of 7.4 years) of whom 26 tested positive for the MeCP2 mutation and 10 were negative, all of whom were correlated with their GI dysfunction. Mutation positive patients had a wide distribution of mutations throughout exons 3 and 4 of the MeCP2 gene. Among these mutation positive patients, all manifested varying degrees of GI dysfunction requiring medical intervention except one subject. Interestingly, this one patient without GI symptoms had a mutation (1161del 6) in the most distal portion of the gene. Patients with more proximal mutations (R168X and R270X) had the greatest number of GI manifestations. The more severe oropharyngeal dysphagia associated well ( $p < 0.05$ ) with mutations (R106Q, T158M and R168X) affecting the proximal portion of the protein suggesting that such mutations may predispose to considerable neuroenteric dysfunction. A similar association between the proximal MeCP2 mutations and severity of neurologic and autonomic dysfunction in RS was noted by Hoffbuhr et al (Neurology, 2001). Conclusion: Severe GI manifestations of RS, such as dysphagia, occur when the proximal portions of the gene are involved reflecting significant brain stem neuronal involvement in addition to the generalized autonomic dysfunction that contributes to reflux and constipation.

**Exploration of the neurobehavioral aspects of Marfan syndrome.** *S. Grimes*<sup>1,2</sup>, *A. Matthews*<sup>1,2</sup>, *J. Bubb*<sup>1,2</sup>, *M. Drumm*<sup>2,3</sup>, *C. Ievers-Landis*<sup>2,3</sup>, *G. Wiesner*<sup>1,2</sup>. 1) Center for Human Genetics, University Hospitals of Cleveland, Cleveland, OH; 2) Case Western Reserve University, Cleveland, OH; 3) Rainbow Babies & Children's Hospital, Cleveland, OH.

Preliminary studies suggest that children with Marfan syndrome (MFS) may be at increased risk for learning problems, attention difficulties, and hyperactivity. Clinical observations also suggest that some may have decreased energy and poor coordination. To explore these possible associations, this study examined parent perceptions of development and neurobehavioral problems in children followed in a multidisciplinary MFS clinic. Fifty parents of children (aged 7-21) with features of MFS and 212 parents of unaffected control children were asked to participate. Parents of 20 affected children and parents of 34 control children completed mailed questionnaires regarding their child's motor and speech development, activity level, attention, and learning problems. Respondents also completed the Child Behavior Checklist, a well-validated measure of competencies and behavioral problems in school-aged children. Affected children were perceived by parents to have significantly more speech ( $p=0.023$ ) and motor deficits ( $p=0.018$ ) and decreased activity levels ( $p<0.001$ ) compared to unaffected peers. The prevalence of learning problems was higher in affected children than in controls, with 30% of affected children reportedly diagnosed with a learning disability. Parents of affected children were more likely to report that their child had attention problems, but they were not more likely to report a diagnosis of Attention-Deficit/Hyperactivity Disorder. These results support previous findings of an association between attention and learning problems in children with features of MFS. Affected children may also be at increased risk for speech and motor delays. These data may help genetic professionals provide appropriate medical and developmental services to affected individuals. Further research is necessary to delineate whether learning and attention problems as well as speech and motor delays represent a behavioral phenotype for MFS.

**Anophthalmia-Microphthalmia in an extended family with consanguinity: further confirmation of Anophthalmia-Plus Syndrome.** *E. Elsobky*<sup>1</sup>, *S.M. Elsayed*<sup>1</sup>, *A. Parsian*<sup>2</sup>. 1) Medical Genetics Center, Heliopolis, Cairo, Egypt; 2) University of Louisville Birth Defects Center, Louisville, KY.

We report an extended pedigree with several members affected with Anophthalmia/Microphthalmia. The proband was a 4 years old female with bilateral extreme microphthalmia with foreshortening of eyelids in all directions, left oblique facial cleft which ended in lower lid coloboma, high arched palate with cleft palate, brachycephaly, and low set ears. Chromosome analysis from blood using high resolution banding was normal 46, XX female karyotype. The younger sister was 3 months old and had bilateral microphthalmia, low set ears and no cleft palate. The parents are first cousins and father's 3 cousins have bilateral microphthalmia without cleft palate or facial clefts. Our pedigree probably is the second one reported so far with microphthalmia-anophthalmia and autosomal recessive mode of inheritance that further confirms anophthalmia-plus syndrome [Fryns et al., 1995].

**Expansion of Wiedemann Beckwith syndrome spectrum, description of a non-viable form discovered at 13 weeks of gestation.** *M. Gerard*<sup>1</sup>, *M. Sinico*<sup>2</sup>, *C. Touboul*<sup>3</sup>, *B. Haddad*<sup>3</sup>, *F. Encha-Razavi*<sup>2</sup>, *J.B. Paniel*<sup>3</sup>, *C. Gicquel*<sup>4</sup>. 1) Medical Genetics, CHIC, Creteil, France; 2) Foetopathology, CHIC, Creteil, France; 3) Obstetrics, CHIC, Creteil, France; 4) Molecular Biology, Pediatric endocrinology, hopital Trousseau, Paris, France.

Wiedemann Beckwith syndrome (WBS) associates macrosomia, macroglossia, omphalocele, visceromegaly, and embryonic tumors. Others frequent features are neonatal hypoglycaemia, facial dysmorphism, frontal nevus flammeus, ear fissures and body hemihypertrophy. Prenatal discovery of WBS is based on the combination of polyhydramnios, fetal macrosomia with macroglossia and parietal defect. A 29-years-old, gravida 2, para 0 was referred. Ultrasound at 13 weeks showed large megacystis and abnormal abdominal wall. The parents were not consanguineous. Karyotype was normal, 46 XY. Termination of pregnancy was elected by the couple. Fetal measurements were between 75P and 90P. A large omphalocele was present, containing small bowel loops, liver left lobe and megacystis. The abdominal wall was very thin, with no evidence of muscle, in a Prune-Belly sequence. Organomegaly were present on liver, brain, kidneys and adrenals. Adrenal cytomegaly was present. Molecular analysis of the 11p15 imprinted region showed an isolated demethylation of KvDMR1 of the KCNQ1OT. The methylation pattern of KvDMR1 was strictly normal in fetus of the same term. Karyotype and methylation analysis of the 11p15 region was normal from both parents. This presentation is particularly severe, non-viable large omphalocele with Prune-Belly sequence. Association of WBS and Prune-Belly sequence has been described in 3 cases. In one familial case, WBS and Prune-Belly sequence were associated in one proband, the other sibling showing BWS and obstructive uropathy (Watanabe and Yamanaka, 1990). Other publications report isolated cases of WBS with complete Prune-Belly sequence (Knight et al., 1980; Silengo et al., 2002). No molecular analysis were reported in these clinical reports. Search for WBS should be conducted for lethal malformative pattern, associating large omphalocele, prune belly sequence signs and adrenal cytomegaly.

**Total asymmetry in a case of severe Silver Russell syndrome.** *L. Bobadilla-Morales<sup>1, 4</sup>, A. Corona-Rivera<sup>1,2,4</sup>, J.R. Corona-Rivera<sup>1,3,4</sup>, Cuerpo Académico Genética Clínica y Médica, CUCS, U. de G..* 1) Laboratorio de Genética Humana, Universidad de Guadalajara, CUCS, Guadalajara, Jalisco, México; 2) Unidad de Citogenética, OPD Hospital Civil de Belén, Guadalajara, Jal., México; 3) OPD, Hospital Civil Juan I Menchaca, Guadalajara, Jal., México; 4) Doctorado en Genética Humana, CUCS, U. de G.

Silver-Russell syndrome (SRS) is characterized by low birth weight, prenatal onset short stature, triangular face, café au lait spots and fifth finger clinodactyly. Besides, unilateral upper or lower limb asymmetries are reported in about 60-85% of the cases with usual variations of around 2.5 cm. Most cases are sporadic, but several modes of inheritance have been proposed. We report a 4.6 yr old boy with severe SRS and total body asymmetry. He was born at term to healthy, non-consanguineous 28-years-old parents. Birth weight was 1600 g and birth length was 42 cm. Intrauterine growth retardation and asymmetry were noticed at birth. He also showed severe psychomotor retardation. Physical examination at 4.6 years of age showed total left asymmetry, left body side length was 92.5 cm and right body side length was 81.5 (11 cm of difference), Right hand and foot were 7.9 cm and 8.4 cm, respectively. Left hand and foot were 8.9 cm and 9.6 cm, respectively. OFC was 47 cm (-3 SD). Triangular left-sided asymmetric face, right iris coloboma, fifth fingers clinodactyly and café au lait spots on limbs were present. On the right side, renal hypoplasia, cryptorchidism and small testicle were observed. The karyotype was 46,XY. Total and severe asymmetry (including the structures of the head, trunk and limbs) has not been previously reported in SRS. Although etiology asymmetry in SRS is unknown, disturbances in the control of symmetry have been proposed. Severe asymmetry in our patient may represent a hemihypertrophy. Chitayat et al. (1988) reported a child with SRS and a hepatocellular carcinoma, a neoplasia more frequent in hemihypertrophy and suggest that common pathologic factors may underlie the development of SRS and hemihypertrophy. Current report supports a probable pathogenic relation between SRS and hemihypertrophy.

**The FG syndrome: report of a large italian series.** A. Battaglia<sup>1</sup>, C. Chines<sup>1</sup>, A. Novelli<sup>2</sup>, J.C. Carey<sup>3</sup>. 1) Inst Child Neurology & Psych, Stella Maris Inst/Univ Pisa, Pisa, Italy; 2) IRCCS-CSS San Giovanni Rotondo Mendel, Univers. La Sapienza, Roma, Italy; 3) Division of Medical Genetics, Dept. of Pediatrics, Univ. of Utah, Salt Lake City, UT, USA.

FG syndrome was first described by Opitz and Kaveggia in 1974, as a rare MCA/MR syndrome occurring only in boys, due to a recessive mutation on the X chromosome. Based on over 50 reported cases, FG syndrome is associated with developmental delay (especially speech), hypotonia, postnatal onset relative macrocephaly, prominent forehead, frontal hair upsweep or whorls, ocular hypertelorism, thin vermilion border of the upper lip, relatively short fingers with broad thumbs and halluces, persistent fetal fingertip pads, anal anomalies, and/or constipation. Major malformations are rare, including pyloric stenosis, anal agenesis, cryptorchidism, hypospadias, inguinal hernias, congenital heart defects. Abnormal EEGs and seizures have been reported in 70% of patients. Brain MRI shows corpus callosum abnormalities, dilatation of lateral ventricles, periventricular nodular heterotopias, mild cerebellar defects, reduced periventricular white matter, and Chiari 1 malformation. Behavior is characterized by ADHD, less developed language and fine motor and executive function skills, whereas visual-spatial abilities seem to be a relative strength. At present, due to an improved awareness, FG syndrome emerges as a rather common MCA/MR syndrome, occurring in boys and girls, presumed to be due to incompletely recessive mutations of genes on chromosome X. Three candidate loci are already at hand, making it possible the cloning of causal gene/s in the next future. We describe 20 patients, referred for evaluation of DD/MR, and diagnosed as FG syndrome. This is the first reported italian series of FG syndrome cases, with particular emphasis on physical, neurological and developmental findings, and natural history. Follow-up ranged between 6 months and 7 years. Subtelomeric analysis, performed in all of them, gave normal results. Experience with our series of patients suggests that FG syndrome may be more common than previously thought and should be routinely considered in the evaluation of children with DD/MR.

**Deletion of paternal 7q21.2-q21.3 is found in some patients with a Seckel-like Syndrome.** *P. Benke<sup>1</sup>, M. Quintero<sup>1</sup>, T. Zuluaga<sup>2</sup>.* 1) Univ Miami Sch Medicine, Miami, FL; 2) Universidad del Valle, Cali, Colombia.

An 11 yr old male with intrauterine growth retardation, short stature, microcephaly, moderate mental retardation, deafness, and facial features resembling the Seckel Syndrome (SS) was found to have an interstitial deletion of chromosome 7q21.2-q21.3. Growth hormone deficiency and a partially empty sella were also found. Experiments with microsatellite markers showed that the 7 q deleted area was 8.5 cM, and extended from D7S1813 to D7S491. The deleted region was paternal, not maternal in origin. An insulin receptor substrate-like gene (IRS3L) in the deleted region may be responsible for much of the short stature seen in this child. A review of previous cases with 7q21.2-q21.3 deletions suggests that some but not all have SS-like features. We suggest that paternal chromosome 7q21.2-q21.3 deletion is a unique form of Seckel Syndrome.

**Familial congenital bilateral perisylvian syndrome: Clinical spectrum and neuroimaging findings.** *I.L. Brandao-Almeida<sup>1</sup>, M.M. Guerreiro<sup>2</sup>, N.F. Santos<sup>1</sup>, C.A. Guimares<sup>2</sup>, S.R. Hage<sup>2</sup>, F. Cendes<sup>2</sup>, L.L. Min<sup>2</sup>, A.M.S.G. Piovesana<sup>2</sup>, M.A. Montenegro<sup>2</sup>, I. Lopes-Cendes<sup>1</sup>.* 1) Department of Medical Genetics, UNICAMP, Campinas - SP, Brazil; 2) Department of Neurology, UNICAMP, Campinas - SP, Brazil.

**Purpose:** The congenital bilateral perisylvian syndrome (CBPS) is frequently caused by polymicrogyria. Clinical features consist of epilepsy in most patients and varying degrees of cognitive deficits. CBPS may have familial recurrence and genetic heterogeneity is likely present. The objective of this work is to correlate clinical and neuroimaging findings in patients with familial CBPS. **Methods:** Family histories were obtained and pedigrees were constructed. Patients were also assessed by neuropsychological tests, language evaluation and high resolution MRI scans. **Results:** We have identified 6 unrelated families with 19 patients with CBPS, 13 males and 6 females. Ages ranged from 3 to 42 years old. Ten patients (53%) had a polymicrogyric appearance of the perisylvian cortex. More detailed imaging analysis revealed that 6 patients had diffuse bilateral perisylvian polymicrogyria, 3 with bilateral posterior parietal polymicrogyria and 1 patient with bilateral fronto-parietal polymicrogyria. Prenatal events were reported in only 3 patients. All patients had similar neurologic dysfunction, mainly primarily pseudobulbar paresis, feeding difficulties in the perinatal period, as well as swallowing and sucking problems. Hemiparesis was present in only 1 patient. None of our patients had epilepsy. Specific language impairment was found in all 19 patients and psychological assessment showed that global cognitive deficit was not present in most patients, although they usually have lower verbal IQ as compared to performance IQ. **Conclusion:** CBPS should be considered as a possible diagnosis in all infants or children with oromotor dysfunction/pseudobulbar signs and developmental language delay. Epilepsy is not a common feature and the most frequent imaging findings in familial CBPS are variable degrees of bilateral perisylvian polymicrogyria. Despite limited verbal IQ, our results support the observation that perisylvian polymicrogyria does not necessarily lead to intellectual disability. Supported by FAPESP.

Program Nr: 606 from 2003 ASHG Annual Meeting

**Wolf-Hirschhorn Syndrome: Clinical and Molecular Cytogenetic Studies in Taiwan.** *J. Hou.* Medical Genetics, Chang Gung Children's Hospital, Taoyuan, Taiwan.

**Background:** Wolf-Hirschhorn syndrome (WHS) is a rare chromosomal disorder characterized by the deletions in the short arm of chromosome 4. There is still very little data on its natural history and wide spectrum of somatic abnormalities, related to the underlying genetic changes. **Methods:** Ten patients (7 females, 3 males) in Taiwan with the 4p- syndrome were followed up for five to fifteen years. The genetic changes were detected by standard cytogenetics (regular G-banding and high-resolution banding) and fluorescence in situ hybridization via painting probe for chromosome 4 and the unique sequences for WHS critical region. **Results:** All of them had severe psychomotor and growth retardation, and a characteristic facies but not changing with time. A total of 9/10 had congenital cardiac defects (most are atrial septal defect); 4/10 had oral facial clefts; 4/10 had a seizure disorder, that tended to disappear with age; 3/10 had agenesis or hypoplasia of corpus callosum; and 3/10 had renal problems. One had sensorineural deafness and one had a bilateral split hands defect. The causes of death in two patients were aspiration pneumonia and end-stage renal failure, respectively. The deleted segments on 4p were from the breakpoints to 4pter: p16.2 (n=2), p16.1 (n=2), p15.33 (n=4), 15.31(n=1), and 15.1(n=1). Karyotype-phenotype correlation study was also performed. **Conclusions:** This experience will help delineate more thoroughly the natural history of WHS, and to obtain better information to answer parents questions in a clinical setting.

**Hypothyroidism in Peters plus syndrome.** *K. Kosaki<sup>1</sup>, R. Kosaki<sup>2</sup>, A. Kamiishi<sup>3</sup>, M. Kawai<sup>4</sup>, T. Takahashi<sup>1</sup>*. 1) Dept Peds, Keio Univ, Tokyo, Japan; 2) Div Med Genet, Saitama Child Med Ctr, Iwatsuki, Japan; 3) Dept Peds, Saitama Chuo Hosp, Saitama, Japan; 4) Dept Ophthalmol, Keio Univ, Tokyo, Japan.

Hypothyroidism has not been previously described in patients with either Peters anomaly or Peters Plus syndrome, with the exception of one report by Jung et al. We report a male patient who exhibited Peters plus syndrome phenotype and hypothyroidism, adding further evidence that hypothyroidism may represent a previously underappreciated feature of the syndrome. The patient was delivered at 38 weeks gestation to a 34 year-old G3P2 woman and a 34 year-old father who were first cousins. The birth weight of 1842 g, length of 41.5 cm, and head circumference of 30.0 cm were all less than the third percentile. He was rhizomelic. At 4 months, he had short palpebral fissures, corneal clouding, mild micrognathia, absence of the antihelix on the right ear, and short neck. Both hands were remarkable for single transverse crease, no flexion crease on the thumb, and clinodactyly of the fifth finger. Ophthalmologic evaluation revealed microcornea and corneal opacity bilaterally. Iridocorneal adhesions were noted in the left eye. Brainstem auditory evoked response revealed bilateral hearing loss. Neonatal screening revealed elevated TSH level. TSH was 33 IU/ml, free T4 was 4.1 pg/ml, and free T3 was 0.19 ng/dl. He was diagnosed as having congenital hypothyroidism and was started on thyroxin and her free T4 and T3 levels were normalized in a week. Ultrasound examination did not reveal thyroid tissue. Consanguinity of the parents in the present case points to a homozygous defect of a single gene responsible for the normal morphogenesis of both the anterior chamber and the thyroid gland, leading to the Peters anomaly and congenital hypothyroidism. We suggest that thyroid function tests are warranted because lack of treatment would potentially aggravate developmental and growth delay that are relatively prevalent among patients with Peters plus syndrome.

**Monozygotic twins with concordant cervical segmentation defects: evidence for genetic basis of Klippel-Feil anomaly.** *R. Kosaki, H. Ohashi.* Div Medical Genetics, Saitama Children's Medical Ctr, Saitama, Japan.

Klippel-Feil anomaly (KFA) is defined as the congenital fusion of two or more vertebrae within the rostral spine. Although it is well accepted that KFA represents a faulty segmentation along the embryo's developing axis during weeks 3-8 of gestation, the origin of this malsegmentation has been a subject of debate. Vascular disruption as well as geneticalteration have both been entertained as possible causes. Relatively popular hypothesis proposes that fetal vascular disruptions in the subclavian artery blood supply during the embryonic period leads to specific patterns of defects in the rostral end of the embryo, collectively referred to as the subclavian artery supply disruption sequence. Familial cases, even if rare, provide support to the concept that the genetic background may contribute to the pathogenesis of KFA. The present report describes diamniotic-monochorionic twins with concordant cervical segmentation defects. Case 1 had a low posterior hair line and a limited range-of-motion for the abduction of the left shoulder joint. A skeletal survey revealed segmentation defects of the spines at the cervical to upper thoracic level. The left scapula was elevated and medially displaced, this condition was diagnosed as a Sprengel deformity. Case 2, twin brother of Case 1 had a short neck with a low posterior hair line but no Sprengel deformity; the range-of-motion his neck and shoulder joints were normal. A skeletal survey revealed a hypoplastic atlas, dense odontoid process, block vertebrae at the C5-C7 level, and a broad clavicle with hypoplasia of the distal ends. To our knowledge, this is the first report of monozygotic twins with concordant cervical segmentation defects. This report supports the existence of a genetic component to the origin of KFA in some cases. Although both twins in the present case exhibited the cervical segmentation defects, the severity of the defect was unequal. Stochastic events during the twinning process could have contributed to the discordance in severity considering that the timing of diamniotic-monochorionic twinning follows that of cleavage when embryonic axes are specified.

**APERT SYNDROME WITH PREAXIAL POLIDACTYLY: A NEW SYNDROME?** *J.M. Mantilla, L.E. Figuera, L. Arnaud, M. Diaz, P. Barros.* Division de genetica.CIBO-IMMS. Guadalajara,Mexico.

Acrocephalosyndactyly type I or Apert syndrome is characterized by craniosinostosis, particular facial dysmorphic features and abnormalities of hands and feet. Although most cases are sporadic, representing new mutations, autosomal dominant transmission with complete penetrance is accepted. The FGFR2 gene is involved and two mutations (Ser252Trp and Pro253Arg) account for most of the patients. We report a case of apparent Apert syndrome with preaxial polydactyly of hands and feet. Rarely, the association of these abnormalities has been reported and, in all the cases, acrocephalopolysyndactyly as the Carpenter syndrome and other diagnostic alternatives have been considered. In 1987, Maroteaux et al. proposed that this association should be considered a new syndrome. Case report: The proposita, a five-month-old female, was born after 38 weeks of an uneventful pregnancy from healthy and unrelated young parents. Her two older brothers are healthy. At birth, she measured 48 cm, weight 3450 gr and the head circumference was 36 cm. She presented the characteristic facial appearance of Apert syndrome: turribrachycephaly, large fontanelles, high forehead, midface hypoplasia, telecanthos, inner epicanthos, prominent eyes, depressed nasal bridge with small parrot-beaked nose, hypoplastic ears and short neck. She had mitten hands with complete syndactyly of 2nd to 5th digits. The feet showed cutaneous fusion of all toes, with very broad first toes. No other abnormalities were observed and the psychomotor development is considered normal to date. X-rays revealed preaxial polydactyly for hands and feet. Cariotype and metabolic screening were normal. Physical findings in the proposita suggest the rare form of Apert syndrome with polydactyly. A review of the reported cases, in order to validate the proposition that this association constitute a new syndrome is made.

**Five new cases of 48,XXXY & 49,XXXXY syndromes & a literature review.** *K. Keppler-Noreuil, A. Muilenburg, S. Patil.* Division of Medical Genetics, Dept Pediatrics, Univ Iowa Hosps & Clinics, Iowa City, IA.

There have been over 50 cases of 48,XXXY and over 100 cases of 49,XXXXY reported with few reported in the recent literature. Although these syndromes are considered to be variants of Klinefelter syndrome, 47,XXY, the clinical findings and natural history are quite different. The purpose of this report is to describe 5 cases with sex chromosome tetrasomy and pentasomy ranging in age from infancy to late adulthood, including a review of their clinical findings, prenatal and natural history with comparison to those cases in the literature.

**CASE 1**, age 30 yrs, was <5th% born at term to a 24 yr old G2P2 mother with IUGR and small placenta. Karyotype 49,XXXXY/50, XXXXXY with 80% having 49,XXXXY. He had IQ 53, and aggressive behaviors. He has multiple skeletal anomalies, microcephaly, hypertelorism, & absent pectoralis muscles. **CASE 2**, 47.5 yrs, was <5th%, born at term in breech SVD to a 30 yr old G3P3 woman. He required NG feeds due to poor feeding. His IQ was <50 with no speech, and impulsive disorder. He has wt and ht ~95%, HC 50%, prominent supraorbital ridging, microphthalmia, hypertelorism, broad nose, prognathism, camptodactyly, radioulnar synostosis, and scoliosis with karyotype 49,XXXXY. **CASE 3**, now 12 yrs, has 48,XXXY/49,XXXXY. He was <5th% born at term to a 25 yr old G2P2 woman. He had microcephaly, suprorbital ridging, prognathism, wide nasal tip, and brachydactyly. He has had SIB, and aggressive behaviors. **CASE 4**, 3 yrs, with 48,XXXY had IQ 73, and normal growth and exam. He was 50th% born at term to 35 yr old G3P3 woman. **CASE 5**, 1 yr, had developmental delay, wt, ht, and HC <5th%, and undescended testis with 49,XXXXY.

These cases highlight the characteristic findings in these two conditions. All but one case in the neonatal period had growth retardation, hypotonia, and poor feeding with absent prenatal complications. Physical features, more prominent with increasing age, involved primarily craniofacial, skeletal and gonadal systems. Lack of dysmorphic findings was noted in 2 of the cases in early infancy. None of these cases had other anomalies, such as cardiac, renal or brain malformations.

**The discordance between clinical findings and molecular genetics in a male patient with some clinical features for Prader-Willi syndrome.** *S. Ninomiya, H. Maruyama, T. Une, M. Kawakami, Y. Yokoyama.* Dept Pediatrics, Okayama Univ. Medical School, Okayama, Japan.

Prader-Willi syndrome (PWS) results from the loss of expression of paternally expressed genes on human chromosome 15q11-q13. Here we report a male patient who had some clinical features for PWS but did not show the paternal deletion or maternal uniparental disomy. Surprisingly, the analysis of microsatellite markers showed only maternal D15S986 locus was deleted and others were biallelic.

**Clinical summaries:** The patient who is now 18-year-old boy was born after 40 weeks of gestation as the offspring of healthy parents with no consanguinity. He showed microcephaly, short neck, webbed neck, low set of ears, down slanting palpebral fissurae, and cryptorchism. He has moderate mental retardation. His hyperphagia has been remarkable since about 12-year-old. His clinical features did not completely satisfy the PWS diagnostic criteria.

**Cytogenetics:** Neither the chromosomal analysis using GTG banding method nor the FISH using a probe of SNRPN showed deletion of 15q11-q13.

**Molecular analysis:** Microsatellite markers within 15q11-q13 and other 15q regions were analyzed by PCR. Primer loci were D15S11, D15S128, D15S122, D15S210, D15S986, D15S1234, GABRB3, D15S165, D15S126, D15S153, D15S211 and D15S127. Each sample was loaded onto denaturing gels and electrophoresis condition was 1,400 V constant volt. The gel was stained by the silver stain kit. It demonstrated biparental inheritance at four loci within 15q11-q13 (D15S11, D15S210, D15S1234 and D15S165) and at four loci distal to 15q13 (D15S126, D15S153, D15S211 and D15S127). Remarkable interestingly only one locus, D15S986, within 15q11.2-q12 showed maternal deletion. The DNA methylation pattern of the patient using by SNRPN probe was normal.

**Conclusions:** The result of maternal deletion at the very small region in this patient does not coincidence with general rule, and suggests that a gene (genes) which is not imprinted but needs biallelic expression exists nearby the deleted region.

**ASSOCIATION OF OPITZ SYNDROME AND PENILE AGENESIS AFTER IVF-ET (ICSI).** *T. Sonoda, H. Nuno.* Dept Pediatrics, Miyazaki Medical Col, Miyazaki, Japan.

Penile agenesis is a rare congenital anomalies that occurs once in every 10 to 30 million births. Though anomalies of the genitourinary tract are the cardinal features of Opitz syndrome, there has been no report presenting the co-occurrence of Opitz syndrome and penile agenesis so far. The male infant proband was born after a 41-week gestation. He was the result of the second pregnancy of healthy and unrelated parents. The father was 58 and the mother was 34 years of age at the time of birth. The facial appearance and intelligence of the parents were normal. None of the family members showed recognizable anomalies. A healthy girl was born by the mothers first pregnancy which was accomplished by *in vitro* fertilization-embryo transplantation (IVF-ET). The proband was also conceived by IVF-ET after intra cytoplasmic sperm injection (ICSI) due to the paternal factor. The proband had a birth weight of 2,356 g, a length of 49.0 cm and a head circumference of 33.0 cm. Because of a peculiar facial appearance and penile agenesis, he was referred to the Department of Pediatrics, Miyazaki Medical College on the 8th day after birth. Physical findings included the following: micrognathia, widows peak, hypertelorism, wide and flat nasal bridge, bulbous nose, shallow philtrum, high arched palate, low-set, post-rotated and cupped ears, left club foot, and hypoplastic nails. The scrotum was normal in appearance and size, and bilateral testes were palpable. The raphe was preset. Position of the urinary meatus was in front of the anus. Movements of bilateral lower extremities were good. Urination and bowel movement were seen periodically. His karyotype was 46,XY. In Opitz syndrome, heterogeneity has been demonstrated. We believe that our case is the autosomal dominant type. Our case probably represents a new mutation, and advanced paternal age may have played a role. Sperms with a certain pathogenic gene may have a low capability of fertilization *in vivo*, but be able to fertilize *in vitro*. Our case adds to the list of midline anomalies previously described in Opitz syndrome. If penile agenesis is a severe form of micropenis or hypospadias, our case also supports the characterization of Opitz syndrome as a developmental defect in the midline field.

**Referral Dx: R/O Turners; Goldenhar; Smith Lemli Opitz; Frontonasal Dysplasia; Post Clinic Dx: 22q11.2 deletion.** *E.H. Zackai, K.L. Russell, J.E. Ming, M.K. Meisenbacher, D.M. McDonald-McGinn.* Div Human Gen/Molec Biol, The Children's Hosp Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA.

It is not surprising that over time, the phenotype of a new syndrome will be expanded to include findings that have been cardinal features of well-established syndromes: primary amenorrhea and the webbed neck of Turners syndrome; pre-auricular skin tags of Goldenhar; post axial polydactyly of Smith-Lemli-Opitz syndrome (SLO) and the median nasal groove of Frontonasal Dysplasia. In fact, these findings often prompt a referral to Clinical Genetics to rule out the classical syndrome. Here we present a series of such patients, all of who were subsequently diagnosed with the 22q11.2 deletion. This emphasizes the importance of a comprehensive approach to the patient and expands the phenotype of the 22q11.2 deletion. To illustrate, patients 1 and 2 were referred to rule out Turner syndrome despite normal 46,XX karyotypes. Patient 1 was a 19-year-old female with short stature, primary amenorrhea and a bicuspid aortic valve. Past medical history revealed a renal ultrasound in childhood, which demonstrated an absent right kidney and no mullerian structures, compatible with Mayer-Rokitansky-Kuster(MRK). We noted short stature, normal breast development, hooded eyelids, a bulbous nasal tip, and learning differences. This prompted 22q11.2 studies, which were positive. The association of MRK and the 22q11.2 deletion has been reported twice before (Huff et al. XXII DW Smith Workshop 2001; Devriendt et al. J Med Genet 34:423, 1997). Patient 2, a 7-year-old was referred because of a webbed neck. Past medical history revealed mild reflux and nasal regurgitation. On physical exam we noted: hooded eyelids, low set ears with thick helices and a prominent nasal root prompting 22q11.2 deletion studies which were positive. Patients with features found in Goldenhar, SLO and Frontonasal Dysplasia will also be discussed. Recognition of the 22q11.2 deletion in these patents is critically important for medical management and recurrence risk counseling.

**Sibs With Hyperekplexia and Status Epilepticus. A New Autosomal Recessive Syndrome.** *C. Vinkler<sup>1</sup>, N. Watemberg<sup>2</sup>, D. Lev<sup>1</sup>, T. Lerman-Sagie<sup>2</sup>.* 1) Inst Medical Genetics, Wolfson Medical Ctr, Holon, Israel; 2) The Metabolic-Neuro-Genetic Clinic. Wolfson Medical Center, Holon, Israel.

Hyperekplexia is a predominantly an autosomal dominant rare disorder often related to a mutation in the alpha 1 subunit of the glycine receptor GRLA1. It is characterized by exaggerated startle response to handling and to noise. Usually increased muscle tone is observed in the neonatal period which improves later in life. Atypical sporadic and autosomal recessive cases were reported with additional variable manifestations. We describe a severe form of hyperekplexia and refractory status epilepticus in two sibs. They were both born to healthy unrelated parents of Ashkenazi-Jewish origin. Their first offspring was a female who was diagnosed with hyperekplexia after her birth. She also had hypotonia and slow motor development. Metabolic evaluation was normal, her muscle biopsy was normal and she had a normal EEG. 80% of the GRLA1 gene was screened for mutation and no mutation was found. When she was 15 months she developed status epilepticus refractory to treatment. She was admitted to the ICU, where she stayed for 17 days before she died. Her brother, was born 4 years later. When he was 3 days old, he was diagnosed as suffering of hyperekplexia. He had normal development. He had status epilepticus for the first time at the age of 11 months. His EEG at that time, was normal. Two weeks later, he had again status epilepticus. He was put on pentobarbital coma and died at the age of one year. The unusual presentation of hyperekplexia together with hypotonia in the girl, and status epilepticus in both children, suggest that this is a new autosomal recessive syndrome, possibly a channelopathy that affects both, spinal cord and brain.

**Chromosome 22q11.2 microdeletion syndrome: Clinical features in 65 Chilean patients.** *G.M. Repetto<sup>1</sup>, M. Aracena<sup>2</sup>, C. Mellado<sup>3</sup>, M. Arriaza<sup>4</sup>, C.P. Astete<sup>2</sup>, T. Aravena<sup>3</sup>.* 1) Genetics, U Desarrollo-Clnica Alemana, Santiago; 2) Hospital Luis Calvo Mackenna, Santiago; 3) Hospital Dr. Stero del Ro, Santiago; 4) Hospital Gustavo Fricke, Via del Mar, Chile.

Chromosome 22q11.2 microdeletion syndrome (del22q11) has variable clinical presentations. Objective: To illustrate the clinical features of Chilean patients with del22q11. Methods: Retrospective review of medical records of patients with FISH-demonstrated deletions from 3 tertiary centers from Santiago and one from Via del Mar, Chile. Results: Sixty-five patients with del22q11 were diagnosed from 1998 to 2002, 53% male. Mean age SD at diagnosis was 37.9 65.2 months, ranging from newborn to 30 years. Ten patients (15.4%) died due to congenital heart defects (CHD), all before 18 months. Height/age was below the 50th centile in 83%, and below the 5th centile in 47%. Forty six individuals (70.8%) had CHD, and 5 others had structural anomalies not requiring surgery. The most common CHD were tetralogy of Fallot (43% of patients with CHD), interrupted aortic arch (IAA)(26%), and VSD (20%). Eight children with IAA had a type B defect and four were type C. Mean ages SD at diagnosis for patients with and without CHD were 21.6 36 and 81.0 101.2 months, respectively ( $p < 0.05$ ). Calcium levels were recorded in 48 patients; 23 had neonatal hypocalcemia, 2 of them required long term therapy. Otolaryngeal problems were present in 30/56 patients, and included submucous cleft palate, velopharyngeal insufficiency, overt cleft palate, and laryngeal membrane, the latter present in 3 newborns. One of the three adults in this series has been diagnosed with schizophrenia. Conclusions: though probably biased by the retrospective nature of the study, the results provide additional useful data for patient counselling and care. Frequency and types of clinical features are similar to published US and European series, but diagnosis tends to occur later, and mortality is higher.

**Arnold-Chiari malformation in Kabuki Syndrome.** *K.L. Russell, E.H. Zackai, J.E. Ming.* Division of Human Genetics & Molecular Biology, Children's Hospital of Philadelphia, Philadelphia, PA.

Structural brain anomalies have only occasionally been described in association with Kabuki syndrome and include hydrocephalus, polymicrogyria, cerebellar and brainstem atrophy, periventricular nodular heterotopia and subarachnoid cyst. Arnold-Chiari malformation, characterized by caudal herniation of the cerebellar tonsils through the foramen magnum, has been described infrequently in association with defined syndromes, and has been reported only once in Kabuki syndrome (McGaughran et al., 2001). We report two children with Kabuki syndrome who also have Arnold-Chiari type I malformation. Patient #1 has a history of cleft palate, developmental delay, a clavicular anomaly, hypodontia and idiopathic thrombocytopenia purpura. On exam, she has epicanthal folds, clinodactyly and prominent fingertip pads. She was diagnosed with Kabuki syndrome at the age of 7 years. At the age of 6 years 10 months, she complained of intermittent headaches and subsequently developed swallowing problems and neck pain. An MRI showed Chiari I malformation with left cerebellar herniation. She underwent surgical decompression. Patient #2 has a history of strabismus, hearing loss, short stature & developmental delay. On exam she has long palpebral fissures, prominent eyelashes, arched eyebrows, mildly protuberant ears with a preauricular pit and fingertip pads. She was diagnosed with Kabuki syndrome at the age of 3 years. She presented at the age of 9 years with a several month history of headache as well as neck pain. She was noted on MRI to have a Chiari I malformation. The incidence of Chiari anomaly may be higher in Kabuki syndrome than published reports suggest. The two patients we report were diagnosed in mid-childhood with Chiari I malformation, and most reported cases of Kabuki syndrome are of younger children. Further, symptoms of Chiari I can be relatively non-specific. Thus, we suggest that this malformation should be considered in children with Kabuki syndrome who present with persistent headache or neck pain. In addition, the diagnosis of Kabuki syndrome could be considered in children with Chiari I malformation if other characteristic findings are present.

**Genetic Studies: A significant component of the Canadian Paediatric Surveillance Program.** *A.M. Summers<sup>1</sup>, A. Medaglia<sup>2</sup>, D. Grenier<sup>2</sup>.* 1) Genetics Program, North York General Hosp, Toronto, ON, Canada; 2) Canadian Paediatric Surveillance Program, Ottawa, ON, Canada.

The Canadian Paediatric Surveillance Programme (CPSP) started in January 1996 under the sponsorship of the Canadian Paediatric Society and the Centre for Infectious Disease Prevention and Control, Health Canada. The purpose of the CPSP is to study relatively rare conditions (<1000 cases/year in Canada) with clear paediatric or public health issues for which there is limited information. Researchers submit protocols which are assessed by a Steering Committee. Approximately ten studies run concurrently, each for 1-3 years. Each month, the CPSP sends out a list of study disorders with a postage paid return envelope to all paediatricians and geneticists in Canada asking them to indicate if they have seen any cases. If a physician has seen a case, he/she is sent a follow-up questionnaire. Compliance is approximately 82%. The initial projects were primarily paediatric, e.g. hemorrhagic disease of the newborn although some, such as progressive intellectual and neurological deterioration, had a genetic component. In January 2001, a project to look at the incidence and prevalence of Smith-Lemli-Opitz syndrome was initiated and that project has now been completed. Currently, there are two genetic projects underway. The first of these is to identify cases of CHARGE association and the second, new cases of Prader-Willi syndrome. A third study on osteogenesis imperfecta has been approved and will start in September, 2003. Of the 21 studies either completed or underway, 7 are genetic or have a genetic component. All of the studies are designed to look at the incidence and/or prevalence of these disorders in Canada but in addition, each study is able to ask specific questions regarding the disorder and has the potential for building up a research base involving interested physicians and families. An added benefit of these projects is that they educate Canadian clinicians about the disorder in question. The CPSP provides an infrastructure which is becoming increasingly utilized by Canadian investigators for research into rare paediatric genetic disorders.

**Unusual digital anomalies in a patient with Brachio-Oculo-Facial Syndrome (BOFS).** *E.J. Ramírez-Lizardo<sup>1,2</sup>, S.E. Totsuka-Sutto<sup>1</sup>, J.R. Corona-Rivera<sup>1,3</sup>, Cuerpo Académico Genética Clínica y Médica CUCS U de G.* 1) Laboratorio de Genética Humana, Departamento de Fisiología, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara. Guadalajara, Jalisco México; 2) Instituto Jalisciense de Cirugía Reconstructiva. SSJ. Guadalajara, Jalisco México; 3) OPD, Hospital Civil Juan I Menchaca. Guadalajara, Jalisco México.

The brachio-oculo-facial syndrome (BOFS) is an entity characterized by craniofacial alterations consisting in branchial anomalies, as auricular pit/sinuses and aplastic or hemangiomatous cervical skin lesions, ear and ocular anomalies and pseudocleft of the upper lip. Other manifestations on the outside of craniofacial area are rare. We report a patient with BOFS and previously unreported digital anomalies. The 3-years-old propositus showed mild disproportionate short stature, dolichocephaly, blond hair, telecanthus, epicanthal folds, lacrimal duct obstruction, short nose, bilateral cleft lip and complete cleft palate, tongue hamartoma, delayed dentition, low-set displasic ears and narrow external auditory canals with apparent aplasia cutis congenita of lateral neck. Limbs were shortened with small hands, A3 brachydactyly and clubbing of fingers. Hallux valgus, mild skin syndactyly, and short second finger toe were also observed. Limb anomalies reported in about 50 BOFS reported cases are rare and includes hand polydactyly, clinodactyly and hipoplastic thumb. To our knowledge, this is the first instance of peculiar digital anomalies in BOFS. Therefore we suggest that these anomalies may be part of this entity.

**Noonan syndrome (NS): PTPN11 gene mutation and phenotype / genotype correlations: the French experience.**

*Y. Sznajder*<sup>1,2</sup>, *B. Keren*<sup>2</sup>, *C. Baumann*<sup>2</sup>, *S. Perreira*<sup>3</sup>, *J. Elion*<sup>3</sup>, *H. Cavé*<sup>3</sup>, *A. Verloes*<sup>2,4</sup>. 1) Clinical Genetics Unit, Pediatric dept, HUDERF, Brussels, Belgium; 2) Clinical Genetic Unit; 3) Genetic Biochemistry Dept; 4) INSERM E9935, Hôpital Robert Debré, Paris, France.

Noonan syndrome (NS) is characterized by facial dysmorphism, short stature, mental retardation and heart defect. Almost 50% of patients (sporadic and familial) carry mutation in PTPN11. PTPN11 gene on 12q24 codes for SHP2, an ubiquitous cytoplasmic protein phosphatase involved in the signalling pathway of several growth factors and cytokines. 95% of the mutation published so far have been identified in exons 3, 7, 8 and 13. About 250 unrelated patients with NS, LEOPARD, Costello, Baraitser-Winter or CFC syndrome diagnosed by a network of clinical geneticists were referred to our institution for PTPN11 analysis. The following data were collected: antenatal ultrasound, feeding difficulties, growth chart, dysmorphism, cardiac status, skeletal-, dermatologic-, ophthalmologic-, auditory- and hematologic anomalies, learning difficulties and pictures over time. Direct sequencing of the key exons of PTPN11 is still going on. At time of submission, 66 out of 158 patients with NS screened for mutations in exons 3, 7, 8, 13 carry a PTPN11 mutation (42.6%). 5 patients with LEOPARD syndrome had mutations in exons 7 (2) and in exon 12 (2), no mutation was identified for CFC (5), Costello (4), Noonan-NF1 (2) and Baraitser Winter (2) syndrome. Among patients carrying a mutation, 11 novel mutations were found. Dysmorphic features were: low set ears (90%), hypertelorism (81%), micrognathia and ptosis (56%). Heart defect were: 59% pulmonic stenosis, 35% ASD, hypertrophic cardiomyopathy 10%. Cryptorchidism was noted for 45% of the males. No phenotype/genotype correlation could be clearly delineated by comparing NS patients with or without mutation except for the cardiac defect (pulmonary valvular stenosis more common and cardiac hypertrophy less common in mutation carriers) as noted in previous studies. We expect to present complete genotype analysis of the cohort for the meeting.

**Mental retardation, facial dysmorphism, skeletal anomalies in first cousins; KBG or a new syndrome? C.**

*Prasad<sup>1</sup>, S. Nikkel<sup>2</sup>, M. Reed<sup>1,3</sup>, S. Sanders<sup>1</sup>, S. Marles<sup>1</sup>.* 1) Department of Pediatrics and Child Health & Biochemistry and Medical Genetics, Children's Hospital, University of Manitoba, Winnipeg; 2) Eastern Ontario Regional Genetics Program, Children's Hospital of Eastern Ontario; 3) Section of Pediatric Radiology.

We present two patients (first cousins) of Caucasian background with short stature, developmental delay, facial dysmorphism, skeletal and dental anomalies reminiscent of KBG syndrome (OMIM #148050). The proposita was delivered prematurely at 32 weeks gestation (birth weight of 1.721 kg). Both parents had developmental delay of unknown etiology. At 5 years, she was below the 10th centile for both height and weight, and her head circumference was 48cm (<3rd centile). The facial features revealed brachycephaly, telecanthus, bilateral epicanthal folds, synophrys, and retrognathia. She had poorly formed nasal bridge and normally placed ears. The dental assessment at 8 years revealed large central incisors, the vertical overbite was 80%, and horizontal overjet was 2.5mm. Numerous primary teeth were present. She had significant bilateral clinodactyly and brachydactyly of her hands, and syndactyly of her 2nd and 3rd toes. The maternal first cousin (male) at the age 5 mos. shared similar facial features to the proposita with bilateral clinodactyly of the hands, without syndactyly of the toes. The proposita had a normal karyotype and subtelomeric deletion studies. Initial skeletal survey showed significantly delayed bone age, questionable fusion anomalies of the spine at the craniocervical junction and the upper thoracic region. The subsequent skeletal survey showed bilateral drumstick 4th digits terminal phalanges. Cone shaped epiphysis were absent. There was diminished carpal height; middle phalanx was mildly hypoplastic in the 5th digits bilaterally and short femoral necks. The skeletal survey on the cousin showed minimal involvement. Though our patients share a few features of the KBG phenotype, we believe that these patients may represent a new entity.

**Missense mutation in the PANK2 gene in a patient with atypical Pantothenate kinase associated**

**neurodegeneration.** *J. Pappas*<sup>1</sup>, *J. Borsuk*<sup>1</sup>, *S. Das*<sup>2</sup>, *H. Bennett*<sup>3</sup>. 1) Clinical Genetic Services, New York Univ, Sch of Med, New York, NY; 2) Department of Human Genetics, The University of Chicago, Chicago, IL; 3) The Stanley S. Lamm Institute for Child Neurology and Developmental Medicine, Brooklyn, NY.

Pantothenate kinase associated neurodegeneration (PKAN), also known as Hallervorden-Spatz syndrome, is an autosomal recessive disorder that usually presents in childhood with dystonia, dysarthria and rigidity. There is a classical form with usual onset of symptoms prior to six years of age and rapid neurodegeneration, and an atypical form with later onset and variable presentation and progression. (SJ Hayflick et al, 2003). We describe a 19-year-old boy (proband) and his 16-year-old sister with the atypical form of PKAN. The proband presented at 8 years of age with dysarthria and excessive drooling. Progressively, he had difficulty closing his mouth with worsening of the dysarthria and drooling, inability to eat solids and regression in his fine and motor abilities. His brain MRI revealed abnormal signal medially at the level of right and left globus pallidus extending to the posterior limbs of the internal capsules. These findings are described as the eye of the tiger usually seen in PKAN. At age 19 his mental abilities were not affected, his mouth remained constantly open with his tongue protruding out and his gait was parkinsonian. His sister presented with learning disabilities, dysarthria and drooling at age sixteen. The parents are first cousins twice removed from the Dominican Republic. The proband was found to have a missense mutation, Y117C, in the homozygous state in the PANK2 gene. This is a non-conservative amino acid change that occurs in a highly conserved region of the PANK2 protein, which has not been described before. This result provides another example of an atypical PKAN patient with a missense mutation in the PANK2 gene. This case represents the first patient with PKAN and a PANK2 mutation from the Dominican Republic. The proband demonstrates that dysarthria progressing to constantly open mouth and protruding tongue can be the main and most dramatic presenting symptoms of atypical PKAN.

**Roberts Syndrome: definition and diagnosis by numerical syndromology.** *H. Vega, M. Gordillo.* Instituto de Genética, Universidad Nacional de Colombia, Bogotá, Cundinamarca, Colombia.

Roberts Syndrome (RS) is a rare autosomal recessive disorder characterized by pre and postnatal growth retardation, phocomelia, craniofacial anomalies and premature centromere separation (PCS). We propose that the RS clinical picture follows a precise pattern defined by five criteria: -The RS facies: microbrachycephaly, malar hypoplasia, hypertelorism, hypoplastic alae nasal, micrognathia and bilateral lip and cleft palate (in severe cases). -Symmetrical mesomelic limb reduction or tetraphocomelia affecting proximal-distal and antero-posterior limb axes. -Characteristic hand pattern with brachydactily that always affects the first finger by agenesis or hypoplasia. The second affected finger is the fifth by clinodactily, and hypoplasia or agenesis. -The clinical picture follows always a cephalocaudal pattern in which the upper limbs are more affected than the lower limbs. -There is a high degree of concordance between the severity of the craniofacial and limb abnormalities. Patients with severe phocomelia and only three digits present prominent eyes, bilateral cleft lip and palate and premaxilla protrusion. Less affected patients present the characteristic Roberts facies without cleft lip and palate, and moderate upper limb reduction and normal lower limbs. Using these criteria we evaluated by numerical syndromology 105 patients reported in the literature in addition to 10 new Colombian patients. We found that all of the 52 patients with PCS were located in the same group in addition to 38 patients that also fulfil all the clinical criteria but were not analyzed for PCS. All of the PCS negative patients were grouped with other cases that also did not fulfil the five criteria. These cases excluded from the RS group can be assigned to other entities such as Zimmer phocomelia, tetraamelia and pulmonary hypoplasia, Splenogonadal fusion limb defect syndrome and TAR. Additionally, we propose two new entities, the Virchow and Freeman Syndromes. We propose RS is not a heterogeneous entity and patients without PCS must be assigned a different diagnosis. Our clinical criteria are a valuable tool for the differential diagnosis of the phocomelias.

**Chromosomal rearrangements in Cornelia de Lange Syndrome: A new case and review of all reported cases. C.**

*DeScipio<sup>1</sup>, M. Kaur<sup>1</sup>, L.G. Jackson<sup>2</sup>, N.B. Spinner<sup>1</sup>, I.D. Krantz<sup>1</sup>*. 1) Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Dept. of Obstetrics and Gynecology, Drexel University College of Medicine, Philadelphia, PA.

Cornelia de Lange syndrome (CdLS), a dominantly inherited disorder, is characterized by facial dysmorphism, limb abnormalities, somatic and cognitive retardation and growth abnormalities. A diagnosis of CdLS relies entirely on clinical findings; the molecular etiology remains unknown. There have been more than thirty reports describing chromosomal abnormalities associated with the CdLS phenotype. Rearrangements have been described in chromosomes 1-5, 7-14, 17, 21, 22, X. With the exception of 3p26, (overlap of the dup3q syndrome phenotype with that of CdLS) there has not been a consistent chromosomal rearrangement involved with CdLS. We report two half sibs with features of CdLS with an unbalanced rearrangement (der(3) t(3;12)(p25.3;p13.3)) inherited from a balanced translocation t(3;12)(p25.3;p13.3) in an unaffected mother. While these children have many features consistent with CdLS (microcephaly, growth retardation, mental retardation, hirsutism, synophrys, anteverted nares, single palmar creases, 2-3 syndactyly of the toes), they also have features seen in other children with rearrangements of 3p and/or 12p. These chromosomal regions may be candidate loci for a CdLS disease gene, however these children may also represent a phenocopy of CdLS. A review of all chromosomal rearrangements associated with CdLS will be presented.

Program Nr: 624 from 2003 ASHG Annual Meeting

**Premature Ovarian Failure and Chromosome Xq28 deletion. A clinical cytogenetic report.** *A.M. Alshafie, M. Abuhenedi, S. Alawadi, L. Bastaki, M. Marafie, M. Krishna, R. Alnaggar.* Obstetric&Gynecology, Kuwait Medical Genetics Center, Kuwait, Kuwait.

The relationship between both primary/secondary ovarian failure(POF) and structural chromosomal rearrangement is well documented. Secondary amenorrhea is mostly recognized as premature menopause in which Xq25-26 deletion has been previously reported. Herein we describe two sisters with premature menopause and deletion Xq28. The two daughters were product of DFC Consanguinous parents. They had normal height, well developed 2ry sexual characters and absence of any stigma of Turner's syndrome. They were infertile and the menopausal symptoms started at 22years for the elder lady and 27years for the younger one. Hypergonadotropic hypogonadism and absence of ovarian follicles have been found. The two sisters had POF, one of them had Xq28 chromosome deletion and the 2nd not yet confirmed. Nevertheless the two daughters possibly have mutation of the assigned gene in the deleted region of chromosome X and we are in favour of recessive mode of inheritance as the parents had DFC consanguinity.

**PROXIMAL 5p TRISOMY IN NEWBORN.** *C. Arioni<sup>1</sup>, A.E. Covone<sup>2</sup>, C. Bellini<sup>1</sup>, C. Cuoco<sup>2</sup>, R. Sacchi<sup>1</sup>, A. Rossi<sup>3</sup>, S. Ciotti<sup>1</sup>, M. Baldi<sup>1</sup>, E. Bonioli<sup>1</sup>, G. Serra<sup>1</sup>.* 1) Dept Pediatrics, Gaslini Inst, Genoa, Italy; 2) Lab Citogenetica, Genetica Molecolare, Gaslini Inst, Genoa, Italy; 3) Dipart Neuroradiologia, Gaslini Inst, Genoa, Italy.

A female infant was born at term of uncomplicated pregnancy to healthy non-consanguineous parents. APGAR scores were 3/7. The patient was referred to our Service on 17th day of life. At birth weight 2,875 g (50th percentile), length 52 cm (90th percentile), head circumference 35.5 cm (75th-90th percentile), anterior fontanelle 0,5 x 0,5 cm, macrocephaly, prominent parietal (fore head), right microphthalmia with skew pupil and iridis coloboma, apparent absence of left globe, low-set ears, bilateral preauricular pits, broad nasal bridge, arachnodactyly, central hypotonia, stridulous cry, poor spontaneous movements. Ocular US scan and Ocular MRI demonstrated right microphthalmos with cystic coloboma of the head of optic nerve which was adherent to the retina, and left microphthalmos with large inferior cyst. Echocardiography showed the presence of large ASD (secundum) with left-to-right shunt, and partial anomalous pulmonary venous return. Kidney tomography showed right pyelectasis. Electromyography showed spontaneous discharge of anterior tibial muscle, possibly related to denervation. Karyotype was 46,XX, der(4)t(4;5)(4qter->4p16::5p12->5pter) (standard Q-banding technique; FISH analysis: probes WCP4, WHS, TEL p/q 1-22 + X and Y, Cri-du-Chat region, and EGR1 x 3). Both parents karyotypes were normal. We could identify the additional chromosomal material as belonging to the short arm of chromosome 5 and therefore the abnormal chromosome 4 present in our patient was deriving from a translocation rearrangement occurred between the two chromosomes. We could establish that this translocation rearrangement occurred de novo in our patient because the karyotype of both parents resulted normal. The patient clinical findings, including facial dysmorphisms, limb abnormalities, cardiac defects, renal and intestinal malformations, mental retardation, fulfilled trisomy 5p clinical picture; our patient presented monolateral coloboma iridis not previously reported.

**Two new cases with a small interstitial deletion within the distal region of chromosome 7q.** B. Faas<sup>1</sup>, P. Poddighe<sup>2</sup>, G. Merks<sup>1</sup>, H. Brunner<sup>1</sup>, D. Smeets<sup>1</sup>, B. de Vries<sup>1</sup>, C. van Ravenswaaij<sup>1</sup>. 1) Department of Human Genetics, UMC St Radboud Nijmegen, Nijmegen, The Netherlands; 2) Department of Clinical Genetics, Rotterdam, the Netherlands.

Interstitial deletions within the distal region of 7q have only rarely been reported. We present two patients with such an interstitial deletion.

Patient 1 is an 11 years old mildly retarded girl who presented with short stature (-3,5 SD), microcephaly (<- 2 SD), arched eyebrows, hypertelorism, upward slanting palpebral fissures, prominent eyes, high nasal bridge, broad mouth, long columella, clinodactyly and short fifth fingers. She had more than 6 cafe-au-lait spots and freckling in the axillary and inguinal region, and was therefore suspected of neurofibromatosis type 1 (NF-1). Patient 2 is an 11 years old moderately retarded girl with short stature, congenital heart anomaly, hypertelorism, a wide nasal bridge, broad mouth and club feet.

Routine karyotyping as well as additional FISH analysis was performed in both patients. Blood from patient 1 was also sent in for DNA diagnostics on NF-1.

Karyotyping showed an apparently identical deletion of 7q32q34 in both patients. FISH analysis, however, proved that the deletions were not identical. The definite karyotypes were: 46,XX,del(7)(q32.2q33) (patient 1) and 46,XX,del(7)(q31.3q32.3) (patient 2). The deletion was excluded in the parents of patient 2; the parents of patient 1 were not available. In patient 1, no known mutations in the NF-1 gene could be detected so far.

We present two patients with a small common deleted region, 7q31.3-q32.2. They strongly resemble a patient recently reported by McGhee *et al.* (Am J Med Genet 2000;93:241-3) who had a t(7;22)(q32;q11.2) and was suspected of Coffin-Siris syndrome. The most prominent features in all three cases are: mild mental retardation, short stature, coarse facial features, hypertelorism, broad base of the nose and a wide mouth with full lower lip and short stubby hands. This indicates that patients with 7q31.3-q32.2 deletions might share a common phenotype.

**A complex and unique chromosomal abnormality.** *E. Elias*<sup>1</sup>, *A. Maleki*<sup>2</sup>, *L. McGavran*<sup>2</sup>. 1) Genetics and Pediatrics, Children's Hospital, Denver, CO; 2) Colorado Genetics Laboratory Denver, CO.

A patient is presented with dysmorphic facies, FTT, and developmental delay. High resolution cytogenetic studies revealed two apparently independent chromosomal abnormalities, with the karyotype: 46,XX,del(1)(p36.3),add(4)(p16.3).

Delivery was at term to a 25 yr old primagravida with BW 2 kg. Subsequent medical course was notable for feeding issues. A genetic evaluation for growth failure and developmental delay occurred at age 18 mo, when she could not crawl, or walk. Language and social skills were at a 10 mo level. Parents were healthy. There was no family history of infertility or fetal loss.

She was a tiny, dysmorphic girl. Weight, height, OFC, and wt/ht were all below the 2nd percentile. Dysmorphic features included hypertelorism, small eyes, prominent pinnae, short philtrum, small mouth, bifid uvula, and brachydactyly. Neurologic exam revealed hypotonia. Echocardiogram was normal. Abdominal ultrasound revealed a solitary kidney.

A small terminal deletion of the distal 1p sub telomere was observed in all cells. Molecular studies showed that the 1p36.3 locus was not deleted. Additional material was present on distal 4p, which was heterochromatic and contained an acrocentric satellite stalk, indicating that add 4 was actually a 4ps. In addition, molecular testing confirmed a deletion of 4p sub telomere sequences, distal to the Wolf-Hirschhorn Syndrome critical region. The maternal karyotype was normal. The father had the identical abnormality of chromosome 4, but normal chromosome 1p.

A patient is presented with a novel chromosomal rearrangement, including deletion of distal 1p, and 4ps. The 4ps abnormality is inherited. As reports indicate that 4ps results in a normal phenotype, her abnormalities are presumed to derive from the de novo 1p deletion. This case helps expand the phenotype of distal 1p deletions, and illustrates the utility of parental and molecular studies in further delineation of complex chromosomal abnormalities.

**Atypical phenotype associated with del 18q21.31: Case study and review of the literature.** *D. Barbouth, M. Zohouri, K.H. Ramesh, L.A. Cannizzaro, V.R. Pulijaal, R.W. Marion.* Departments of Pediatrics and Pathology, Albert Einstein College of Medicine and the Children's Hospital at Montefiore, Bronx, New York.

First described by de Grouchy et al. in 1964, the 18q- syndrome is a well-described disorder that combines small stature, mental disability, hypotonia, craniofacial malformations (including midface hypoplasia, flat philtrum, carp-shaped mouth, broad nasal bridge), hearing loss, ophthalmic anomalies and minor abnormalities of the hands. We describe a 21 year old patient with a deletion of chromosome 18q21.31 qter region and the clinical features of this patient are atypical of 18 q- syndrome. The 5 pound 11 ounce product of a 38 week gestation, was born by vaginal delivery to an 18 year old woman, G4P1021 (SAB x 2) and a 21 year old man after an essentially uncomplicated pregnancy. At birth, he was found to have hypospadias, a bicuspid aortic valve, and talipes equinovarus. Work-up in the newborn period included CT scan of the head, EEG, metabolic screen, and metaphase karyotype, all of which were normal. On the basis of the clinical features, a diagnosis of Smith-Lemli-Opitz syndrome was made. At 21, the patient presents with repaired hypospadias and mild mental retardation. He also has hypothyroidism, which was diagnosed at age 20, and a seizure disorder, which was first noted at age 18, both of which are being treated. On physical exam, his height is at the 25th percentile and weight is at the 50 percentile; he has mild dysmorphic facial features including facial asymmetry, high arched palate and submucous cleft, hypertelorism, up-slanting palpebral fissures, raised nasal bridge, high placed ears and scoliosis. High resolution chromosome studies reveal a de novo terminal deletion of the long arm of chromosome 18 at band 21.31. Further characterization of this deletion by FISH using whole chromosome painting and subtelomeric probes for chromosome 18 confirm that the deletion is terminal and there is no rearrangement of 18q subtelomeric region i.e, 46,XY,del (18) (q21.31) ish del(18) (q21.31) (WCP18x2, TEL18q11). Molecular studies are in progress seeking genotype-phenotype correlation of this patients 18q deletion and atypical 18q- phenotype.

**ATR-16 syndrome: Further delineation of the phenotype.** *R.J. Lin, A.M. Cherry, L. Hudgins.* Division of Medical Genetics, Departments of Pediatrics and Pathology, Stanford University School of Medicine, Stanford, CA.

Alpha-thalassemia retardation-16 (ATR-16) syndrome is associated with microdeletions involving the terminal 2 Mb of 16p13.3. It is characterized by  $\alpha$ -thalassemia and mild to moderate mental retardation. The other features that have been described include hypertelorism with down-slanting palpebral fissures, epicanthic folds, strabismus, a broad flat nasal bridge, talipes equinovarus, and an asymmetric chest. Although many of the individuals with ATR-16 syndrome have unbalanced translocations, there have been a few reported cases with pure monosomy of 16p. However, the small number of cases has only allowed for limited clinical characterization. We report the case of a 17 year-old Hispanic female with the above findings, along with cleft lip and palate, high frequency hearing loss, ventriculoseptal defect, short stature, congenital onychodystrophy, microcephaly, and craniosynostosis of the right lambdoid suture. High resolution chromosome analysis was normal; however, subtelomeric FISH probe study demonstrated a terminal deletion of 16p13.3 and no evidence of a translocation. The abnormality was not present in the mother; the father was unavailable for study. This is the sixth reported case of pure monosomy of 16p13.3, and expands the clinical phenotype. The previously reported cases are of a milder phenotype than our patient, with deletions in the range of 750-2000 kb. ATR-16 syndrome is most likely a contiguous gene syndrome. Several genes have been identified in this region including the  $\alpha$ -globin genes. Further analysis of the extent of the deletion in our patient will be helpful in determining whether a larger region of monosomy leads to a more severe phenotype. However, other factors may also contribute to a more severe phenotype including chromosomal position effects, parental imprinting, and genetic background.

**Molecular characterisation of ring chromosome 22.** *A.R. Jeffries<sup>1</sup>, S. Curran<sup>2</sup>, P. Sham<sup>2</sup>, H. Fiegler<sup>3</sup>, K. Woodfine<sup>3</sup>, N.P. Carter<sup>3</sup>, J. Powell<sup>1</sup>.* 1) Neuroscience, Institute of Psychiatry, London, United Kingdom; 2) SGDP Research Ctr, Institute of Psychiatry, London, United Kingdom; 3) Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

Ring chromosome 22 (r22) is a rare human cytogenetic abnormality. Individuals with ring chromosome 22 exhibit a phenotype of global developmental delay with severe delay or absence of speech associated with more variable features including hypotonia, minor facial anomalies and 2-3 toe syndactyly. In common with other ring chromosomes, r22 is assumed to arise from breakage and subsequent fusion of both chromosome arms to generate a ring chromosome with concomitant loss of short and long arm sequences. Loss of short arm sequences is unlikely to be of clinical importance but hemizyosity for critical genes on the long arm of chromosome 22 has phenotypic implications. We have characterised the long arm breakpoints in 34 families, the largest reported collection of r22 patients. Microsatellite analysis was carried out to determine the 22q breakpoints and the parental origin of the r22. From 27 individuals, 11 were of maternal and 16 of paternal origin. Four individuals were also found to be mosaics. 22q DNA loss varied from less than 69 kb up to 10.1 Mb (0.15% to 21% of the total chromosome length). Visual inspection of the breakpoint positions suggested that these were clustered. We conducted a simulation study to formally test this hypothesis. Genotypes for each chromosome were simulated, using marker positions obtained from Ensembl and heterozygosity values from Genethon. An algorithm was used that scored the number of pairs of simulated chromosomes that overlapped in the interval that contained the breakpoint i.e. between the first homozygous deleted and heterozygous markers. This simulation was repeated ten thousand times and the score distribution plotted. The score of the real data was within the highest 0.3% of this empirical distribution providing significant evidence for clustering of breakpoints. We are now fine mapping the breakpoint positions using a chromosome 22 tiling path genomic microarray.

**A novel genomic rearrangement of 7q11.23 in multiple unrelated families with Williams-Beuren syndrome.** *L. Osborne<sup>1</sup>, M. Haddad<sup>2</sup>, M. Schachow<sup>2</sup>, M. Li<sup>2</sup>, J. Skaug<sup>2</sup>, R. Lokkesmoe<sup>3</sup>, K.W. Gripp<sup>4</sup>, E. Thompson<sup>5</sup>, L. Perez-Jurado<sup>6</sup>, S.W. Scherer<sup>2</sup>.* 1) Medicine, University of Toronto, Toronto, ON; 2) Genetics & Genomic Biology, The Hospital for Sick Children, Toronto, ON; 3) Genetic and Molecular Medicine, Kingston General Hospital, Kingston, ON; 4) Clinical Genetics Service, Women's & Children's Hospital, North Adelaide, SA; 5) Medical Genetics, DuPont Hospital for Children, Wilmington, DE; 6) Ciencias Experimentals i de la Salut, Universidad Pompeu Fabra, Barcelona, Spain.

Williams-Beuren syndrome (WBS) is commonly caused by the heterozygous deletion of a 1.6 Mb region of chromosome 7q11.23, but a small proportion of individuals with symptoms do not carry this deletion. We had previously identified an inversion of the WBS region, INV-I, which was associated with WBS phenotypes in a few cases, and was present in approximately 30% of transmitting WBS parents where it was proposed to predispose the chromosome to subsequent deletion. To determine whether inversions of the WBS region are common in families with a typical (deleted) or atypical (no apparent deletion) WBS proband, we have studied the 7q11.23 region using three-colour interphase FISH in more than 50 families. In one family with two WBS children with deletions, we found that in both affected siblings the deletion had occurred on an inverted chromosome 7 inherited from their father. This is the first example of a multiplex WBS family carrying a parental inversion. In six individuals with features of WBS we have now identified a second inversion, INV-II, with completely different breakpoints from INV-I. Whilst INV-I had endpoints located within the duplicated segments flanking the WBS common deletion, INV-II has endpoints within apparently single copy regions: one within the deletion region between WBSR1 and CYLN2, and the other distal to the deletion region, between SCYA26 and TMPIT. The clinical features of the individuals with this rearrangement vary but include developmental delay and sometimes other features of WBS such as hypersensitivity to sound, anxiety, characteristic facies, hoarse voice and musculoskeletal abnormalities. Interestingly, in some individuals INV-II is present on 50% of chromosomes examined, whilst in others it is present on only 30-40% of chromosomes examined. This raises the possibility of mitotic instability of the WBS region and the presence of a threshold effect for normal chromosomes, below which clinical features start to manifest. Further analysis of these rearrangements and identification of the exact breakpoints will help to explain the molecular mechanism for the inversion and the associated phenotypes.

**A Novel Case with 49 chromosomes: Implications of trisomies other than 13,18,21 and X chromosomes in live borns.** *S. MOVVA<sup>1</sup>, M. KUMAR<sup>1</sup>, S. NAJEEB<sup>1</sup>, P.S. MURTHY<sup>1</sup>, Q. HASAN<sup>1,2</sup>*. 1) Bhagvan Mahavir Hospital And Research centre Hyderabad-500 004, AP, INDIA; 2) Kamineni Hospitals, L.B. Nagar, Hyderabad-500 068, AP, India.

A ten-month-old boy, with delayed developmental milestones was referred to our department for cytogenetic evaluation. The child was 3rd in birth order delivered vaginally to a consanguineous couple at full term. At birth the only phenotypic abnormalities noticed were low set ears and wide spaced eyes. A suspicion of some abnormality arose at 5 months when the child failed to attain head holding. There is H/o recurrent respiratory tract infections with failure to thrive. On detailed evaluation, the child appeared conscious, irritable, moderate pallor, no cyanosis/icterus, no general lymphadenopathy or Pedal oedema. He weighed 5.5kgs(<50th percentile), length 67cm, arm span 58cms, US/LS 48/37 (1:1.3), Normal hair line, anterior fontanel 3/4cms, PF closed, hypertelorism with interpupillary distance of 4cm (95th percentile), depressed nasal bridge, high arched palate, normal philtrum, low set ears, short stubby fingers with normal dermatoglyphics. Bilateral undescended testis with phallic length of 12mm. Respiratory system showing bilateral crepts with wheeze, CVS-S1, S2 no audible murmurs, hepatosplenomegaly, and D/Q assessment developmental age of 5 months. CNS- no focal deficit. Family and antenatal history were unremarkable apart from an earlier male sibling who had multiple skeletal deformities and died at the age of 5months (medical reports not available). Lymphocyte culture revealed a karyotype of 49, XXXY + Trisomy10. There is no evidence of mosaicism. Buccal epithelium showed 16% cells with a double X chromatin body. Parental age and karyotype doesnt provide any evidence for the cytogenetic compliment of the proband. Extensive search in publications and internet didnt provide any information on live borns with a high chromosome compliment apart from cases of myelodysplastic syndrome and hematological malignancies. The mild phenotypic dysmorphisms seen in this child is surprising and he represents a novel case of a survivor with double trisomy, other than trisomies involving 13,18, 21 and X.

**Sotos syndrome with submicroscopic deletion of 5q35.** *K. Kurosawa<sup>1</sup>, Y. Igarashi<sup>1</sup>, T. Yamamoto<sup>1</sup>, M. Masuno<sup>1</sup>, K. Imaizumi<sup>1</sup>, N. Matsumoto<sup>2</sup>, Y. Kuroki<sup>1</sup>.* 1) Kanagawa Children's Med Ctr, Yokohama, Japan; 2) Department of Human Genetics, Nagasaki University School of Medicine, Nagasaki, Japan.

Sotos syndrome is characterized by pre- and postnatal growth acceleration, advanced bone age, developmental delay and characteristic facial appearance. Recently haploinsufficiency of NSD1 gene at 5q35 is suggested to be the major cause of Sotos syndrome, especially for two-thirds of Japanese patients. We have evaluated 11 individuals (7 boys and 4 girls) with Sotos syndrome who are between age 4 months and 16 years, in order to better define the Sotos syndrome of deletion type. We review the clinical features and the spectrum of congenital anomalies. Conventional chromosome analysis did not detect visible rearrangements of 5q35. The diagnosis was based on the clinical features and FISH analysis with RP1-118M12 PAC clone, involving the NSD1 gene. Of the 11 patients, all have moderate to severe mental retardation. Mean birth weight was 3315.427g, length 49.525cm, OFC 35.519cm. Prenatal overgrowth (>3,500g) was noted in 4 out of 11. Paternal ages are 29.852, maternal ages 28.145, which is not significantly different from those of general population in Japan. As the CNS involvements, hypoplasia of corpus callosum is found in 2, in addition to the ventricular dilatation. 7 out of 11 have developed epilepsy. The age of the onset of seizures is early childhood. The seizures include grand mal and focal seizures. Congenital heart defects, involving hypoplastic left ventricle, A-P window, tetralogy of Fallot, and PDA were repaired in early infancy. Vesicoureteral reflux was noted 6 in 11. Postnatal overgrowth was noted only in 2 cases, which is the most significant difference compared with those of Sotos syndrome without submicroscopic deletion of 5q35. The information of the natural history of Sotos syndrome with submicroscopic deletion of 5q35 is suggested to be important for health practitioner and patient families for caring the disorder.

**Mosaicism for trisomy 17q in a female with normal intelligence.** *T. Mononen, R.L. Meltoranta.* Dept Clinical Genetics, Kuopio Univ Hosp, Kuopio, Finland.

We report a 13-year-old female with mosaic trisomy 17q. She presented with short stature (height about -2 SD) and a 3-cm leg length discrepancy. She had uneven, patchy skin pigmentation on the trunk, arms, and thighs that was not strikingly apparent due to her light complexion. She had dysmorphic features e.g. telecanthus, a low nasal bridge, a short anteverted nose, thick lips, posteriorly rotated ears, fleshy ear lobes, a low nuchal hairline, and fetal finger pads. There was no major malformations. Echocardiogram and renal ultrasound examination were normal. She had reached her developmental milestones but she was hypotonic and nonathletic. Her school performance was good.

Chromosome analysis of peripheral blood lymphocytes showed a normal female karyotype. Skin biopsies were taken from several separate sites. The G-banding and FISH analysis revealed the karyotype to be: mos 47,XX,+del(17)(p11.2)/46,XX in all the skin specimens studied. The percentage of the abnormal cells averaged 50% (60/120 cells). Parental karyotypes were normal.

This case illustrates the importance of obtaining skin fibroblast chromosome analyses even in patients with normal peripheral blood chromosomes who have multiple minor malformations and slight alterations in growth, body asymmetry, or skin pigmentation.

**Decreased Cholesterol synthesis as a possible aetiological factor in malformations of Trisomy 18.** *W. Lam<sup>1</sup>, J. Kirk<sup>2</sup>, N. Manning<sup>3</sup>, W. Readon<sup>4</sup>, R.I. Kelley<sup>5</sup>, D. FitzPatrick<sup>1,6</sup>.* 1) Dept Clinical Genetics, Univ Edinburgh, Edinburgh, United Kingdom; 2) Department of Clinical Biochemistry, Royal Hospital for Sick Children, Edinburgh; 3) Department of Paediatric Pathology, Sheffield Childrens Hospital, Sheffield; 4) National Centre for Medical Genetics, Our Ladys Hospital, Dublin; 5) Kennedy Krieger Institute, Johns Hopkins Medical Institutions, Baltimore, MD USA; 6) Medical Research Council, Western General Hospital, Edinburgh.

Cholesterol is a 27-carbon mono-unsaturated sterol and the major sterol in mammals. It serves as an important structural lipid of cell membranes. Cholesterol is also the precursor of all known steroid hormones. In human cells, most cholesterol is from de novo synthesis. Over the past decade, several human malformation syndromes have been identified as disorders of cholesterol biosynthesis. Trisomy 18 is a well-known malformation syndrome characterized by intrauterine growth retardation and multisystemic involvement including skeleton, cardiac, central nervous system and urogenital abnormalities. At present how the extra chromosome cause malformations is unknown. We report a series of 3 neonates and 6 fetuses with trisomy 18 in whom the cholesterol level in serum at birth or in amniotic fluid at 16-18 weeks gestation were abnormally low. The proband was born with IUGR and multiple congenital abnormalities suggestive of trisomy 18, which was confirmed by cytogenetics. However a subsequent report of the cholesterol level was 0.2 mmol/L (control 1.7 +/- 0.2 mmol/L) associated with a 7 dehydrocholesterol level of 0.1 mol/L (control 1-2 mol/L),  $p < 0.001$ . Two other trisomy 18 neonates with abnormally low levels of cholesterol were subsequently identified. In addition we found the mean level of cholesterol in 6 mid-trimester amniotic fluids from fetuses with trisomy 18 to be significantly low compared to the level of cholesterol in control amniotic fluids from normal fetuses 16.9 mmol/L (control 33.9 +/- 11.7 mmol/L),  $p < 0.05$ . We propose that hypocholesterolemia is a common characteristic of trisomy 18 and may have a role in causing the malformations. The recurrent finding of an abnormally low serum cholesterol level suggests a disruption of cholesterol biosynthesis.

**CHROMOSOME 1 IN THE GENETIC CLINIC.** *B. Kousseff, G. Jervis.* Pediatrics & Genetics, USF Regional Genetics Prog, Tampa, FL.

Chromosome 1 contains 10% of the human genome and it is not known for predilection to chromosome anomalies. Numerous reports have addressed a variety of chromosome 1 abnormalities but laboratory/clinic correlations are lacking. Between 1/2/82 and 12/31/02 through busy (2002 patient volume 2,797) prenatal and pediatric clinics at University of South Florida Genetics clinic 42,795 probands/families were evaluated. Forty-three had anomalous chromosome 1. Twenty-four had translocations, three had complex chromosome rearrangements (CCR) with 3 or more break points, seven had *inv(1)* and 2 had *del 1p*. One each had *ins(1p)*, *der(1)* secondary to questionable translocation, mosaic trisomy 1 (Clinical Genetics 1988: 33; 73-77), *del 1q*, *dup 1q*, *dup 1p* and *1qh+* initially thought to represent *inv(1)* leading to prematurity of 32 weeks and fatal outcome due to congenital diaphragmatic hernia, respectively. (The *1qh+* heteromorphism was not considered an anomaly and was not kept track of). One of the *inv(1)* pat was with interstitial deletion *15q* and was evaluated through the prenatal clinic as were 18 other probands. Two were evaluated for family history of anomalous chromosome 1 and the remaining 22 through the pediatric clinic. Among the 24 translocations 5 were paternal, 9 maternal and 10 *de novo*. Translocation *1;4* was encountered four times, *t(1;11)* and *t(1;6)* three times each and *t(1;5)* two times. However the break points were different, without predilection to a particular break point. The anomalies in the 5 unbalanced translocations varied as did the break points. The 3 CCR had 3, 4, and 5 breakpoints respectively and anomalous chromosome 1 was the only chromosome shared. The seven *inv(1)* were 5 pericentric and 2 paracentric. Only one was *de novo*; it was paracentric. The rest were 3 paternal and 3 maternal. Altogether the probands were 35 Caucasians, 7 Hispanics and 1 African-American. There was no explanation for the under representation of African-Americans. The study illustrated the wealth of cytogenetic, clinical, and outcome findings of patients with anomalous chromosome 1 in busy tertiary genetic centers.

**Molecular analysis of a translocation t(17;20)(q25;q13) associated with Silver-Russell syndrome.** *F. Matthes, M. Ayala-Madrigal, I. Hansmann, D. Schlote.* Martin-Luther-University Halle, Institute of Human Genetics, Halle, Germany.

A severe Silver-Russell syndrome (SRS), a heterogenous disorder mainly characterized by lateral asymmetry, pre- and postnatal growth retardation and other morphological abnormalities, ascertained in a girl with a translocation t(17;20)(q25;q13) inherited from her phenotypically not affected father, was reported by Ramirez-Dueñas et al. (1992). Here we report the molecular analysis of the breakpoint region on both chromosome 17q25 as well as 20q13 based on specific BAC / PAC clone contigs established for these regions. FISH analysis using proband metaphase chromosomes in order to map the molecular breakpoint in 17q25 determined several BAC and PAC clones giving signals on both chromosomes 17 and derivative 17 thus establishing a refined clone contig for the region of interest. According to the mutation analysis of a 3,5 cM interval on chromosome 17q25 for hereditary neuralgic amyotrophy (HNA; Meuleman et al., 2001) we started mapping several candidate genes onto this contig. One of the genes (SEC14L1) could be localized nearby the region of interest by PCR approach. Further refining the existing clone contig by STS content mapping and sequencing the BAC / PAC insert ends resulted in defining the breakpoint within 81,5 kb in the 5'-region of SEC14L1. Corresponding analysis of the breakpoint region on chromosome 20 led to the identification of a PAC clone spanning the breakpoint including one exon of the gene PTPRT, encoding a receptor protein tyrosine phosphatase, now being considered another candidate gene for Silver-Russell syndrome. Subcloning PAC and BAC subfragments and using these clones for Fluorescence-in situ-hybridization experiments facilitates refining the molecular breakpoint on chromosome 20. RFLP analysis in order to identify aberrant fragments specific for the translocation is furthermore being used to localize the molecular breakpoints hence giving novel information on the genetic background of the development of Silver-Russell syndrome.

**Balanced translocation 6q/21q predisposing to multiple miscarriages and trisomy 21 in a three generation**

**pedigree.** *S.H. Morelli*<sup>1,2</sup>, *A.M. Meloni-Ehrig*<sup>1</sup>, *B. Issa*<sup>1</sup>, *T.J. Pysher*<sup>3</sup>, *Z. Chen*<sup>1</sup>. 1) Dept Pediatrics, University of Utah, Salt Lake City, Utah; 2) Dept of Neonatology, Utah Valley Regional Medical Center, Provo, Utah; 3) Dept of Pathology, University of Utah, Salt Lake City, Utah.

We present a case of trisomy 21 with a balanced 6q/21q translocation who died at 10 days of age from complications of prematurity. This 1201 gm female infant was born to a 24 year old G3 P1121 mother. Pregnancy was complicated by rupture of membranes at 20 wks without re-accumulation of fluid. She remained on bedrest and went into labor and progressed to spontaneous vaginal birth at 27 weeks gestation. At birth the infant had positional deformities of her extremities and face consistent with oligohydramnios, but she also had facial features of Down syndrome. Her chromosome analysis revealed a 47, XX, t(6;21)(q25.3;q22.3), +21 karyotype. She lived for 10 days with severe complications including probable hypoplastic lungs, bilateral pneumothoraces, small membranous VSD, intraventricular hemorrhage, and PDA. Autopsy confirmed the findings: physical features of Down syndrome, bronchopulmonary dysplasia, tracheitis, bilateral germinal matrix hemorrhage with extension into right ventricle. Family history revealed extensive fetal loss in three generations: 5 women had a total of 24 miscarriages, many in the second trimester. One male had a prolonged, 15-year period of infertility. At least one miscarriage was shown to have trisomy 21. There is a first cousin once removed to the proband with Down syndrome. Preliminary studies confirm that the mother of the proband carries the balanced 6q/21q translocation. Additional studies of the family are pending. There are few reports of balanced translocations in individuals who also have trisomy 21. Balanced 6q translocation has been previously described as a predisposing factor for miscarriages. The mechanism may be that unbalanced gametes are formed by unfavorable adjacent 1 or 2 segregation leading to the high risk for miscarriage in this family. The 3 individuals with trisomy 21 in the extended family and the young maternal age in the case presented support that the balanced 6q/21q translocation may also predispose to non-dysjunction and trisomy 21.

**Molecular and cytogenetic characterization of an interstitial deletion of 14q [del(14)(q12)] in a child presenting with microcephaly and frontal lobe cortical dysplasia.** *G. Macintyre*<sup>1</sup>, *B. Lo*<sup>2</sup>, *D. Kamnasaran*<sup>1</sup>, *S. Meyn*<sup>2</sup>, *D.W. Cox*<sup>1</sup>. 1) Department of Medical Genetics, University of Alberta, Edmonton, Canada; 2) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, Canada.

Interstitial deletions or translocations of 14q11-12, rarely reported, are associated with diseases including benign hereditary chorea (NKX2.1), tetramelic mirror image polydactyly (MIPOL1), oligodontia (PAX9), holoprosencephaly (Kamnasaran *et al*, 2001, *Am J Med Genet* 102:173) and schizophrenia (NPAS3; Kamnasaran *et al*, 2003, *J Med Genet*. 40:325). We have characterized a de novo deletion in a boy who presented in infancy with microcephaly (<3rd %), hypotonia and developmental delay. Soft dysmorphisms at eight months included prominent metopic suture, bitemporal narrowing, upslanting palpebral fissures, thin upper lip and posteriorly rotated ear. MRI imaging (1 year) showed a solitary cyst in the left lateral ventricle and subtle cortical pachygyria involving the frontal lobes. A hearing test and ERG exam were normal. Focal seizures developed at 1.5 years. Chromosomal analysis by G-banding revealed a karyotype of 46XY,del(14)(q12q13.1). Parental karyotypes were normal and family history was non-contributory. FISH analysis with BAC genomic clones and STS marker analysis on flow sorted chromosomes were then used to ascertain the extent of the deletion, which spans at least 2Mb and encompasses D14S80 and D14S262. The deletion is outside a locus we have recently defined for holoprosencephaly. A similar, but not identical, deletion has been reported. The two cases share some features including generalized hypotonia and microcephaly. A third reported case, with a potential small overlapping deleted region, has pachygyria, suggesting the location of a gene influencing brain structure. Two previously described genes reside within or adjacent to the deleted region, as well as several predicted genes supported by ESTs. FOXG1 is a transcription factor that may play a role in brain and telencephalon development and NOVA1 is a neuron-specific RNA-binding protein.

**Hypoplasia of portal vein and intrahepatic tumor in a patient with Wolf-Hirschhorn syndrome.** *T. Kosho<sup>1,5</sup>, H. Hisazumi<sup>2</sup>, K. Yakubo<sup>3</sup>, K. Matsushima<sup>4</sup>, T. Tsuchiya<sup>1</sup>, T. Ichikawa<sup>1</sup>, K. Mori<sup>1</sup>, K. Maeyama<sup>1</sup>, Y. Fukushima<sup>5</sup>, A. Tsuji<sup>1</sup>, H. Ohashi<sup>6</sup>, S. Sato<sup>1</sup>.* 1) Dept of Pediatrics, Saitama Municipal Hospital, Saitama, Japan; 2) Dept of Radiology, Saitama Municipal Hospital; 3) Dept of Obstetrics and Gynecology, Saitama Municipal Hospital; 4) Dept of Clinical Laboratory, Saitama Childrens Medical Center, Saitama, Japan; 5) Div. Clinical and Molecular Genetics, Shinshu University Hospital, Nagano, Japan; 6) Div. of Medical Genetics, Saitama Childrens Medical Center.

Wolf-Hirschhorn syndrome is a chromosomal disorder caused by microdeletion of the short arm of chromosome 4. It shows variable clinical manifestations such as severe growth and developmental retardation, characteristic facies, cleft lip and palate, skeletal abnormalities, cardiac defect, urogenital defect, and convulsion, but liver complications have not been reported. We present a female neonate with Wolf-Hirschhorn syndrome exhibiting hypoplasia of portal vein and intrahepatic tumor. At 30 weeks of gestation, amniocentesis was performed for intrauterine growth retardation and the chromosomes were 46,XX,del(4)(p16.1). At 37 weeks of gestation, she was born with growth retardation (birth weight 1572g (-3.0SD), height 39.6cm (-3.5SD), and OFC 29.6cm (-1.5SD)), characteristic face, cleft lip and palate, preaxial polydactyly, and hip joint contracture. Ultrasonography showed no flow in portal vein and reversed flow in dilated splenic vein. Enhanced 3D-CT demonstrated severe hypoplasia of portal vein and dilatation of splenic vein draining into inferior vena cava (IVC) via left renal vein. Mild hyperammonemia and galactosemia were noted. Thus, extrahepatic shunt from portal vein to IVC via splenic vein and left renal vein was confirmed. Enhanced 3D-CT also showed low density tumor (1.3cmx1.6cm) in the right lobe of the liver with significant hypervascularity. Hemangioendothelioma or nodular hyperplasia was suspected, while hepatoblastoma was excluded due to no elevation of serum AFP. This is the first report of liver complications of Wolf-Hirschhorn syndrome and careful evaluation of liver might be recommended in patients with this syndrome.

**Cluster of Down's syndrome cases in Belarus - possible consequence of Chernobyl accident.** *G. Lazjuk, I. Zatsepin, R. Khmel.* Dept Monitoring Cong Malform, Inst Hereditary Disease, Minsk, Belarus.

In Belarus Institute for Hereditary diseases a National register of congenital malformations is running since 1979. The data base contains information about 3104 cases of Down's syndrome (DS), more than half of them are verified cytogenetically. The study performed on liveborns and stillborns as well as cases aborted for genetic reasons with DS revealed a significant increase of monthly prevalence at birth in January 1987 (2.5 as compared to 1.2 for 1987 and 1.1 for the whole period under study). The largest increase was marked in the most affected by the Chernobyl accident Gomel oblast where the population obtained the largest doses. The association with external dose due to the increase of the exposure dose rates by one and even two orders of magnitude in majority of regions of Belarus during first days of the accident as a result of Chernobyl plum passage is supposed. According to the created model the pick of conception followed by the birth of DS children coincide the time of the highest exposure dose rates, thus the time around the conception is supposed to be the critical one. The obtained results are in accordance with the experimental data. The studies performed on laboratory mice (Tease, C., 1982; Evans, H.J., 1986) showed the most radiosensitive stage of oogenesis for induction of nondisjunction of chromosomes during this very short period (meta-anaphase of meiotic divisions). The temporal deviations of maternal age isn't responsible for the revealed pike. The average age of the women who gave birth to DS children was approximately the same as for other periods and in Gomel oblast it was even somewhat lower (23.1 years as compared to 29.3 years). The prenatal diagnostics couldn't be the cause as well, because it was introduced only in early 90-s. The results need additional experimental investigations.

**A case of balanced reciprocal translocation t(15q13;17q23) and repeated miscarriage due to Meckel syndrome. S. Tayel.** Clinical Cytogenetics Unit, Department of Anatomy, Alexandria Faculty of Medicine, Alexandria, Egypt.

Peripheral blood karyotyping was performed to a 42-year old lady with recurrent fetal loss. A balanced translocation between 15q and 17q was diagnosed with the karyotype of 46,XX,t(15q;17q)(17pter -->17q23 :: 15q13 --> 15qter,15pter--> 15q13 :: 17q23-->17qter). FISH analysis using WCP for chromosomes 15 and 17 confirmed the translocation. The lady had 3 mid-trimester abortions with fetal ultrasonography illustrating Meckel syndrome features in the form of occipital encephalocele, polycystic kidneys, cardiac defect, growth retardation with decreased fetal movement, single umbilical artery, ascites, oligohydramnios and breech presentation. Cases with balanced translocation usually have normal phenotype. The abnormal phenotype in such patients could be explained by the presence of microdeletion at the break points or the gene position effect. This case adds more proof to the known MKS gene locus (17q21-q24) and it may extend the genetic heterogeneity of Meckel syndrome beyond the 11q13 (MKS2), and 8q24 (MKS3) to 15q13. Translocation carriers with such history (high age, and recurrent abortion) could be advised to get benefit from ICSI and PGD using specific FISH probes to select a normal embryo.

**Molecular cytogenetic characterization of multiple intrachromosomal rearrangements of chromosome 2q in a patient with Waardenburg syndrome and other defects.** *S. Shim*<sup>1</sup>, *H.E. Wyandt*<sup>1</sup>, *E.H. Zackai*<sup>2</sup>, *D.M. McDonald-McGinn*<sup>2</sup>, *A. Milunsky*<sup>1</sup>. 1) Ctr Human Genetics, Boston Univ, Boston, MA; 2) Children's Hospital of Philadelphia, Philadelphia, PA.

Waardenburg syndrome type I (WS-1) was diagnosed clinically in a 6 year old boy with bilateral sensorineural deafness, lateral displacement of the inner canthi, a bulbous nasal tip, synophrys and cryptorchidism. Neither parent showed signs of this disorder and the family history was negative. In addition, he had a lumbar spina bifida with hydrocephalus shunted on day 2 of life, and severe mental retardation with a head circumference at the 5%tile. Initial karyotyping was reported as normal. Because of the WS-1 features, attention was focused on the PAX 3 location in 2q, at which time a de novo paracentric inversion of 2q23-q37.1 was noted (Zackai et al, *Am J Hum Genet* 57:A106, 1995). Subsequent high-resolution chromosome analysis indicated a complex rearrangement involving regions 2q31-q35 and 2q13-q21. Whole chromosome painting and high-resolution CGH yielded negative results for any translocation, duplication or deletion of any chromosome segment. Sequencing of the PAX 3 gene yielded no detectable mutation. FISH studies with human BAC clones revealed 5 breakpoints in chromosome 2q resulting in 2 paracentric inversions and 1 insertion, the karyotype being interpreted as 46,XY,der(2)inv(2)(q13q21)inv(2)(q21q23)ins(2)(q23q33q35). In this extremely rare chromosomal rearrangement, the FISH result showed a breakpoint at 2q35 being proximal to and without involvement of the PAX 3 gene. While further studies continue, possible interpretations include involvement of a regulatory gene(s) for PAX 3 and other genes at the other breakpoints related causally to the spina bifida and mental retardation.

**Familial pure partial trisomy of chromosome segment 7q32-q34: further delineation of phenotype.** *J.A. Phalen<sup>1</sup>, G.H. Scharer<sup>2</sup>, A. Maleki<sup>3</sup>, E. Sujansky<sup>2</sup>.* 1) Child Development Unit, The Children's Hospital, Denver, CO; 2) Genetics and Metabolism Services, The Children's Hospital, Denver, CO; 3) University of Colorado Health Sciences Center, Denver, CO.

We report a mother and son with pure trisomy 7q32-q34. To our knowledge only 3 prior cases are reported. All were secondary to insertion of maternally-derived chromosomal material. The propositus was a 13-month-old mildly dysmorphic male with global developmental delay born to a 23-year-old mother with moderate mental retardation. Prenatal ultrasound showed unilateral hydronephrosis, which resolved by six months of age. He was born vaginally to unrelated parents at 38 weeks gestation weighing 3.04 kg. At age 13 months his height was 69 cm and weight 8.36 kg (both < 2nd percentile), wt-to-ht ratio 53rd percentile; OFC 46.5 cm (50th percentile). Dysmorphic features included positional occipital plagiocephaly, prominent mid-face with micrognathia, slightly low-set ears, hypoplastic nose, long philtrum, high-arched palate, and single palmar crease. He had truncal hypotonia with peripheral hypertonia. MRI of the brain and an EEG were normal. Mother had a long thin face, high frontal hairline, slightly low-set ears, long philtrum, and high-arched palate. High-resolution chromosome analysis from blood lymphocyte cultures of the child and mother, analyzed by standard GTG-banding at the 450-650 band range, revealed a 46,XY and 46,XX karyotype, respectively, both with extra chromosomal material on chromosome 7, at position 7q32.3q34. FISH on metaphase cells using a whole chromosome paint probe for chromosome 7, a 7q subtelomeric probe, and a probe specific for the 7q31 region confirmed duplication of segment 7q32-34. Common to all 5 reported patients are normal gestational and birth data, mental retardation or developmental delay, minor skull abnormalities, a prominent mid-face, high frontal hairline, low-set ears, long philtrum, and abnormal palate. Unique to our family are absence of eye, hand, and foot anomalies. Unlike 2 adult brothers previously reported, this mother had no seizures. Thus, it appears that patients with pure trisomy 7q32-34 may be less severely affected than those previously reported.

**Delayed diagnosis of chromosome abnormalities in non-dysmorphic individuals with epilepsy.** *J.L. Tolmie<sup>1</sup>, S. Macleod<sup>2</sup>, A. Mallik<sup>3</sup>, J.B.P. Stephenson<sup>2</sup>, M.E. O'Regan<sup>2</sup>, S.E. Zuberi<sup>2</sup>.* 1) Duncan Guthrie Institute of Medical Genetics, Yorkhill Hospitals NHS Trust, Glasgow, U.K; 2) Fraser of Allander Neurosciences Unit, Royal Hospital for Sick Children, Glasgow; 3) Regional Clinical Neurophysiology Department, Southern General Hospital, Glasgow.

It is well known that there is increased risk of epilepsy associated with chromosomal abnormality syndromes such as Miller-Dieker syndrome, 1p36 deletion and Angelman syndrome, conditions that present with profound developmental delays, dysmorphism and congenital malformation. However, it is not so well known that an epilepsy phenotype may be the presenting feature that leads to diagnosis of a chromosome abnormality. We identified 11 patients who each had a clinical presentation with an epilepsy phenotype. Five patients had sex chromosome abnormalities, four patients had ring chromosome 20, one had ring chromosome 14 and one had a balanced reciprocal translocation. Most likely, in these patients the absence of dysmorphism explained delayed diagnosis of the chromosome abnormality and accounted for inappropriate investigations and treatments, especially in patients with ring chromosome 20, three of whom underwent epilepsy surgery evaluation before the chromosome diagnosis was made. We conclude that chromosome studies should not be delayed in children who have learning difficulties dating from or after the onset of epilepsy, nor should they be delayed in individuals with atypical non-lesional epilepsy, even in the absence of learning difficulties.

**Trisomy 8 Mosaicism Syndrome.** *A.L. Torres-Rodriguez<sup>1</sup>, M. Diaz-Garcia<sup>1</sup>, G. Garcia-Sanchez<sup>1</sup>, C.F. Martinez-Cruz<sup>2</sup>, S.G. Juarez-Garcia<sup>1</sup>, F. Castillo-Lorca<sup>1</sup>.* 1) Departamento de Genetica.Instituto de la Comunicacion Humana/Centro Nacional de Rehabilitacion, Mexico, D.F; 2) Servicio de Comunicacin Humana, Departamento. de Seguimiento Pediatrico.Instituto Nacional de Perinatologia, Mexico, D.F.

Complete trisomy 8 occurs in 0.8% of spontaneous pregnancy loss. Mosaic trisomy 8 is a well-described syndrome, characterized by rather specific combination of congenital malformation. And a pattern of minor physical abnormalities. Over 75 cases have been reported. This is more frequent in males. The face is characterized by large, pear shape nose, evert lower lip, small mandible, high palate, unusual ear morphology, and long neck. The features are extremely variable but may include; mental retardation, skeletal anomalies, congenital heart defects and kidney malformations. A significant number of cases have ocular manifestations. The most commonly reported in the literature have been corneal abnormalities and strabismus. We present a 4-year-old male child with phenotype of mosaic trisomy 8. The patient present low anterior hairline, narrow and flat forehead, arched eyes brown, broad nose, protuberant lips, micrognathia, flat cleft, small fingers and toe joints resulting camptodactyly in hands and feet. The patient shows the characteristic deep plantar creases in feet. The thorax is long and narrow, present scoliosis, supernumerary nipple vestige in both axillas and had interventricular septal defect. The audiometric study was normal. Chromosome analysis was done in lymphocyte and fibroblast culture, the result of the karyotypes was 46;XY in lymphocyte and 46,XY/47,XY+8 (12% / 88% respectively) in fibroblast. The literature to make mention which the percentage in the expression cellular are more variable. The distribution of the additional chromosome in different tissues is highly variable too. In this case the trisomy 8 only was observed in fibroblast cells.

**A Syndrome of partial trisomy of 16q and partial monosomy of 18p.** *R. Siddiqui*<sup>1,3</sup>, *E. Pergament*<sup>2</sup>, *J.B. Ravnan*<sup>4</sup>, *L. Guohui*<sup>4</sup>, *J.G. Davis*<sup>3</sup>. 1) Medical Genetics, Memorial Sloan Kettering, New York, NY; 2) Northwestern University Medical School Chicago, IL; 3) New York Weill Cornell Medical Center, NY; 4) Genzyme Genetics.

Partial trisomy of chromosome 16q and monosomy of chromosome 18p are uncommon chromosomal disorders. Individuals who present with both partial trisomy of chromosome 16q and partial monosomy of chromosome 18p are rare. We report a second case of a female child who presents with this combination.

NJ was born at 39 weeks of gestation to unrelated parents ages 32 each. The couple had difficulties with conception. Conception was achieved using clomiphene and intrauterine insemination. A second trimester ultrasound revealed mild hydrocephalus. Cytogenetic studies using GTG banding performed on amniocytes, obtained at 27th weeks of gestation, showed 46,XX,add(18)(p11.2). FISH analysis of cord blood cells after birth demonstrated that the additional genetic material was derived from a translocation between chromosomes 16 and 18; ish der(18)t(16;18)(wcp16+,wcp18+). Chromosome analyses of both parents revealed normal karyotypes.

At birth, NJ weighed 9 lbs. 2 oz (97th percentile) and was 21 inches long (97th percentile). Physical examination at birth revealed an infant with asymmetric skull; dysmorphic facial features, including down slanting palpebral fissures, small nose with anteverted nares, low set ears, grooved philtrum, recessed chin; overlapping fingers: 2 over 3 and 5 over 4, and bilateral dislocated hips. The patient was hypotonic and had clonus at both ankles. Echocardiogram showed an atrial septal defect and brachial artery stenosis. There were no other congenital anomalies. NJ developed gastroesophageal reflux requiring Nissen fundoplication. Subsequently, NJ has enjoyed good general health. Her hypotonia persists. She now exhibits developmental delay.

This is the second case report of an individual who presents with multiple congenital anomalies and developmental delay who has both partial trisomy for long arm of chromosome 16 and monosomy for short arm of chromosome 18 with multiple congenital anomalies.

**6q subtelomeric deletion: Case report and review of the literature.** *D.A. Stevenson, K.M. Dent, J.C. Carey, Z. Chen, N. Longo.* Pediatrics, University of Utah, Salt Lake City, UT.

We report on a girl with an abnormal hybridization pattern for the subtelomeric fluorescence in-situ hybridization (FISH) probe panel showing deletion of the long arm telomeric region of chromosome 6. All other subtelomere DNA probes showed normal hybridization patterns. Metaphase cells analyzed from cultures of peripheral blood revealed a normal female chromosome complement at the 650-band level. Whole chromosome painting probes for chromosome 6 showed both homologs 6 to be entirely painted in 10 metaphase cells analyzed. In both parents, FISH using locus-specific probes for the subtelomeric region of 6q showed no evidence for a deletion as seen in the patient. Clinical findings include: developmental delay, seizures, hypoplasia of the corpus callosum, dextrocardia, unusual dimpling of knees and elbows, and minor facial anomalies. We are aware of only two other reports of a 6q subtelomeric deletion. Retinal abnormalities have been associated with distal deletions of 6q but the cases with subtelomeric deletions of 6q did not have retinal abnormalities. Genes responsible for such retinal abnormalities seen in other terminal 6q deletions may be proximal to 6q27. These three cases support the use of subtelomeric FISH probes in patients with developmental delays and minor anomalies with apparently normal karyotypes or balanced chromosome rearrangements. As more cases are reported we may be able to establish a discrete phenotype and natural history to aid in counseling.

**Non-syndromic mental retardation: Identification of candidate X-chromosome loci.** *E. Sherr*<sup>1</sup>, *P. Cotter*<sup>2</sup>. 1) Dept Neurology, Univ California, San Francisco, San Francisco, CA; 2) Cytogenetics and Molecular Genetics, U.S. Labs, Irvine, CA.

Profound cognitive impairment is a significant health issue, affecting approximately 1-3% of children worldwide. Recent progress has been made in identifying a few genetic mutations underlying this disorder, yet for the majority of these patients, no diagnosis or specific treatment options are available. We have evaluated a young girl with significant cognitive impairment and difficulties with adaptive functioning who has an X chromosome inversion. Her developmental progress was delayed, more in language than in gross or fine motor milestones. Her exam is significant for no dysmorphic features, mild lower extremity hypertonicity and awkward gait. Comprehensive metabolic testing did not reveal any significant abnormalities and a high resolution MRI did not detect a structural CNS cause for her difficulties. A WISC-III demonstrated a full scale I.Q. of 54. Cytogenetic analysis (>550 bands) detected a pericentric inversion, (46,X, inv (X) (q13.1; q26.3). To evaluate this further, we have undertaken FISH analysis to more precisely determine the breakpoints of this inversion with the aim of identifying candidate genes as the cause of her mental retardation. Genomic BACs spanning both Xq13 and Xq26 were labeled by nick translation with fluorescent dUTP and hybridized to metaphase spreads of a control and the patient. BAC 177A4 hybridized within the inversion, thus defining the proximal breakpoint centromeric to Xq13.1. BAC 112H23 also hybridized within the inversion, defining the distal breakpoint telomeric to Xq26.3. Replication studies in lymphocytes demonstrated that the inverted chromosome was the early replicating. Ongoing FISH analysis will enable precise localization of these two breakpoints and identification of candidate mental retardation genes.

**Paternal origin of LMNA mutations in Hutchinson-Gilford progeria.** *M.R. D'Apice<sup>1</sup>, R. Tenconi<sup>2</sup>, M. Biervliet<sup>3</sup>, I. Mammi<sup>2</sup>, J. van den Ende<sup>3</sup>, G. Novelli<sup>1</sup>.* 1) Biopathology, University of Tor Vergata, Rome, RM, Italy; 2) Dept. of Pediatrics, University of Padua, Italy; 3) Department of Medical Genetics, University of Antwerp, Belgium.

Hutchinson-Gilford progeria syndrome (HGPS; OMIM #176670), is a rare and progressive disorder characterized by multiple anomalies, including slowed growth, facial disproportion with prominent eyes, receding mandible and protruding ears, hair loss, osteoporosis and thin, parchment-like skin with depleted subcutaneous fat deposits. HGPS is caused by sporadic mutations in the lamin A gene. At least 5 different mutations within the LMNA gene have been found in patients with HGPS. The mutation responsible for the majority of cases of HGPS is a de novo single-base substitution C-to-T resulting in a silent gly-to-gly change at codon 608 within exon 11 (G608G). The G608G mutation causes the activation of a cryptic splice site within exon 11 of the LMNA gene, resulting in the production of a protein product that deletes 50 amino acids near the C terminus. We analyzed the parental origin of LMNA G608G mutation detected in our sporadic cases of HGPS. Paternal and maternal haplotypes were established by segregation analysis of SNP markers at the previously reported in LMNA gene in position 861T/C (ex5), position 1338T/C (ex7); position 1698C/T (ex10). The genomic region encompassing the exon 10, intron 10 and exon 11 was PCR amplified for each patient and the resulting 1200-bp PCR product was cloned and sequenced as described above. By using the polymorphism data, we established the phase of the mutation, and therefore also its origin. We found that the germline mutation occurred in all four analyzed cases, on the paternal haplotype and concluded that de novo mutations in HGPS occur exclusively on the paternally derived 1 chromosome. This is in accord with the observation of certain human genetic conditions where incidence of new mutations increases with the age of the father. We confirm this hypothesis in progeria, since an age significantly higher (p<0.001) than expected paternal age was also observed in our families and provided molecular evidence that HGPS new mutations originate during spermatogenesis.

**FGFR2 and severe anomalies: phenotype-genotype correlation.** *R.E. Dugan<sup>1</sup>, E. Bawle<sup>2</sup>, K. Kalche<sup>3</sup>, A. Rozelle<sup>2</sup>, A.D. Gilbert<sup>1</sup>, R. Romero<sup>3</sup>, A. Johnson<sup>1</sup>.* 1) Obstetrics and Gynecology, Wayne State University, Detroit, MI; 2) Children's Hospital of Michigan, Detroit, MI; 3) Perinatal Research Branch, National Institute fo Health, Bethesda MD.

Background: The FGFR-related craniosynostosis syndromes are associated with coronal synostosis, midfacial hypoplasia with proptosis, and variable hand and foot anomalies. Traditionally, these disorders have been defined by clinical observation and variable severity; however, molecular observation has shown that these disorders are in fact allelic conditions that can share identical mutations. Case Report: MT is a 26 Y.O. G7P5025 of Hispanic and Caucasian ancestry. Targeted 2-D and 3-D ultrasounds at 36 wks revealed cloverleaf skull, exophthalmia, bowing of long bones, large great toe, shortened, flexed thumbs, and disorganized cervical-thoracic spine. A male neonate was born at 39 wks with cloverleaf skull, remarkable proptosis, and severe midface hypoplasia. Skeletal survey revealed 2-3 finger syndactly, adducted thumbs, synostosis of the elbow with fusion of the proximal radius, ulna and distal humerus, knees fixed at 80-90 , and anterior curvature of the tibia. The vertebrae appeared abnormal on CT. Additional findings included imperforated anus, hypospadias, VSD, and PDA. Postnatal DNA analysis revealed a TYR340Cys mutation in exon 9 of FGFR2. The child is alive at 1y.o. Conclusion: This is a case of severe craniosynostosis with additional skeletal anomalies associated with a suspected de novo FGFR2 mut. In a study of Antley-Bixler syndrome (ABS), 4/7 cases with an indentifiable FGFR2 mutation involved the creation of a new Cys residue. One of the first reported cases of ABS associated with a FGFR2 mut involved the creation of a Cys residue and severe spinal dysraphism, a finding not of ABS but similar to those reported here. These mutations have been reported with severe Pfeiffer syndrome. An unpaired Cys is thought to result in ligand-independent activation yielding a severe phenotype. Given the allelic nature of these mutations, the spectrum of disease and new therapeutic interventions, determining prognosis based on FGFR2 analysis remains difficult, especially in a prenatal setting.

**9q34.3 Terminal Deletion in Three Unrelated Patients: A new MCA/MR Syndrome.** *N. Matsumoto*<sup>1, 2</sup>, *M. Iwakoshi*<sup>3</sup>, *N. Okamoto*<sup>4</sup>, *N. Harada*<sup>1, 2, 5</sup>, *T. Nakamura*<sup>6</sup>, *S. Yamamori*<sup>6</sup>, *H. Fujita*<sup>6</sup>, *N. Niikawa*<sup>1, 2</sup>. 1) Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; 2) CREST, Japan Science and Technology Corporation, Kawaguchi, Japan; 3) Nishinomiya Municipal Wakaba-en, Nishinomiya, Japan; 4) Department of Planning and Research, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan; 5) Kyushu Medical Science Nagasaki Laboratory, Nagasaki, Japan; 6) Research Department, Mitsubishi Kagaku Bio-Clinical Laboratories, Tokyo, Japan.

A de novo 9q34.3 terminal deletion was found in three unrelated patients by all-subtelomere FISH analysis and/or subtelomere-specific microarray CGH. The 9q34.3 deletion in two patients was simply terminal, while that of the other was due to an unbalanced translocation, der(9)t(9;6)(q34.3;p25)de novo. All patients presented with common characteristic craniofacial abnormalities including flat round face, light thin soft hair, arched eyebrows, hypertelorism, short nose, anteverted nostrils, open carp mouth, thick lower lip, protruding tongue, and pointed chin. Severe mental retardation was recognized in all of them. Cardiac anomaly and hydronephrosis were observed in two cases, and obesity was found in one case. We propose that the 9q34.3 deletion is a distinct MCA/MR syndrome.

**Deletion of GPR35 Gene On 2q37.3 May Account For Albright Hereditary Osteodystrophy Like Phenotype : Defining Further Genetic Variants Within and Around GPR35 Gene.** *J. Hoo<sup>1</sup>, A.E. Shrimpton<sup>1,2</sup>, B.R. Braddock<sup>1</sup>, L.L. Thomson<sup>1</sup>, C.K. Stein<sup>1,2</sup>.* 1) Dept Pediat, SUNY Upstate Med Univ, Syracuse, NY; 2) Dept Pathology, SUNY Upstate Med Univ, Syracuse, NY.

Patients with a cytogenetic diagnosis of terminal band 2q37 deletion can be divided into two groups on the basis of their clinical features. The first group comprises the majority of cases and is associated with significant growth and mental retardation but rather nonspecific physical features. The second group, a minority of less than 10 reported cases, clinically resembles Albright hereditary osteodystrophy (AHO) / pseudohypoparathyroidism (PHPT) and has only mild to moderate mental retardation. We report three patients of group 2. Cytogenetic FISH studies followed by molecular analyses showed that the gene for G protein-coupled receptor 35 (GPR35) on 2q37.3 is deleted in all three patients. We postulate that absence of this particular G protein receptor gene may account for the phenotypic resemblance to the Albright hereditary osteodystrophy. We also believe that the deletion of GPR35 is responsible for the entity Brachydactyly - Mental Retardation syndrome (OMIM #600430). We have further defined several polymorphic variants within the coding region and flanking regions of GPR35 gene, which could potentially be useful for rapid detection of GPR35 gene deletion. We recommend that every patient with AHO/PHPT phenotype should undergo 2q subtelomeric FISH screen and further molecular study on the GPR35 gene as necessary.

**Subtelomeric deletions detected in 6.7% of mr/mca patients by multiplex ligation-dependent probe amplification (MPLA).** *Y. van Bever, L. Rooms, E. Reyniers, R. van Luyck, J. Wauters, J.J. van den Ende, R.F. Kooy.* Department of Medical genetics, University of Antwerp, Antwerp, Belgium.

Subtelomeric rearrangements are estimated to be responsible for 5-10% of cases of unexplained mental retardation. Despite the high frequency, routine methods to screen for these rearrangements are scarce.

We screened 75 patients with unexplained mental retardation and either a familial history of mental retardation or facial dysmorphism/multiple congenital anomalies (MCA) or both, for a subtelomeric deletion using multiplex ligation-dependent probe amplification (MPLA). A subtelomeric deletion was identified in 5 patients. Two patients had a 1pter deletion, (the most frequently described deletion), whereas a 1qter deletion, a 21qter and a 3pter were found once in three different patients. The patient with the 3pter deletion also had a duplication of the subtelomeric region of chromosome 11q as detected by FISH analysis. The two patients with 1pter deletion both had MR, hearing impairment and dysmorphic facies, compatible with reports of patients described with a similar deletion. The 1qter deletion patient had MR, epilepsy, growth retardation, a single kidney, and hypospadias. The patient with a 21qter deletion had severe PMR, truncal obesity, a lax and somewhat translucent skin, strabismus, fleshy nose and thick lips. The patient with the 3pter deletion had a phenotype compatible with the 3p- syndrome: severe MR, growth retardation, microcephaly and dysmorphism.

Our conclusions are that subtelomeric screening is an important tool in diagnosing patients with unexplained MR and that MLPA is a sensitive method to perform this screening.

**Quantitative PCR (QPCR) screening detects atypical 22q11.2 microdeletions in adults with features of 22q11.2 deletion syndrome negative by diagnostic FISH testing.** *R. Weksberg*<sup>1,2,3</sup>, *J.A. Squire*<sup>4</sup>, *E. Chow*<sup>5</sup>, *A.S. Bassett*<sup>5</sup>. 1) Div Clinical & Metabolic Genetics, Dept Paediatrics, Hosp Sick Children, Toronto; 2) Dept Molecular & Medical Genetics, Univ Toronto, Toronto; 3) Research Inst, Hosp Sick Children, Toronto; 4) Ontario Cancer Institute and Depts of Lab Medicine, Pathology and Medical Biophysics, Univ Toronto, Toronto; 5) Centre for Addiction and Mental Health, and Dept of Psychiatry, Univ Toronto, Toronto.

Chromosome 22q11.2 deletion syndrome (22q11.2 DS) encompasses a heterogeneous group of disorders. Clinical features include palatal anomalies, cardiac malformations, learning and psychiatric disorders. The majority of 22q11.2 deletions are 3Mb or 1.5Mb long and can be detected by a standard diagnostic FISH assay using the TUPLE1 (Vysis) probe. However, a significant number of patients with features of 22q11.2 DS are atypical in that they do not exhibit hemizyosity with this diagnostic FISH assay. A number of reports of such patients describe atypical microdeletions that are unique within this 3Mb region. To map and screen for atypical hemizygous microdeletions in adult syndromal patients negative for 22q11.2 deletion by diagnostic FISH with TUPLE1 we have used real time QPCR with fluorescent dye SYBR Green 1 chemistry and the ABI Prism 7700 sequence detection system. This screen of the 22q11.2 region uses 9 primer pairs that are evenly spaced within the commonly deleted 3Mb region and 5 primer pairs in the flanking regions. We have tested 3 normal controls, 15 adults with deletions and 2 adults without deletions by standard diagnostic FISH testing. Our QPCR data demonstrate for these 2 latter patients a microdeletion in 22q11.2 which does not include TUPLE1. Hemizyosity in these 2 cases was confirmed with FISH probes from the region of hemizyosity detected by QPCR. Currently we are analyzing 17 adults with features of 22q11.2 DS who are negative by standard FISH testing. Preliminary data shows that at least one with tetralogy of Fallot has a small atypical microdeletion detectable by QPCR. This approach will ameliorate diagnosis of individuals with 22q11.2 DS and will generate high resolution deletion maps elucidating the genetic basis of 22q11.2 DS.

**Uniparental disomy of the X chromosome in a female with recessive X-linked ichthyosis.** *C. Thauvin-Robinet Jr<sup>1</sup>, P. Caillier<sup>2</sup>, G. Vaillant<sup>3</sup>, A. Donzel<sup>4</sup>, V. Cusin<sup>1</sup>, B. Favre<sup>2</sup>, F. Huet<sup>5</sup>, J.R. Teyssier<sup>4</sup>, F. Mugneret<sup>2</sup>, L. Faivre<sup>1</sup>.* 1) Centre de Genetique, Hopital d'Enfants, Dijon, France; 2) Laboratoire de Cytogenetique, Hopital Le Bocage, Dijon, France; 3) Service d'Endocrinologie, Hopital Le Bocage, Dijon, France; 4) Laboratoire de Genetique Moleculaire, Faculte de Medecine, Dijon, France; 5) Service de Pediatrie 1, Hopital d'Enfants, Dijon, France.

Recessive X-linked ichthyosis is a genetic disorder of keratinization characterized by generalized desquamation of large, adherent, dark brown scales. Extracutaneous manifestations include corneal opacity and cryptorchidism. Abnormal cutaneous scaling results from steroid sulphatase gene (STS) deficiency. The STS gene is mapped to the Xp22.3-pter region, which escapes X chromosome inactivation. Complete deletions of the STS gene have been found in up to 90% patients, using FISH studies. Here, we report the case of a karyotypically normal female affected with a maternally inherited recessive X-linked ichthyosis as a result of a homozygous deletion of the STS gene, detected by FISH studies. The patient presented with mild ichthyosis (located on face and arms) and labia minora hypertrophy. PCR analysis of microsatellite polymorphic markers spanning the STS gene region consistently demonstrated a single allele in favor of maternal X chromosome isodisomy. PCR analysis of other microsatellite polymorphic markers spanning the entire X chromosome in order to delimitate the extension of the isodisomy showed partial maternal short arm of X chromosome isodisomy. The manifestation of mild to severe symptoms of recessive X-linked disorders in female carriers results usually from Turner syndrome or skewed X inactivation with preferential inactivation of the X chromosome carrying the normal allele. In the literature, only one female case with uniparental disomy of the entire X chromosome is reported, in association with Duchenne muscular dystrophy.

**Prenatally diagnosed isodicentric Yp.** *H. Bruyere*<sup>1</sup>, *M.D. Speevak*<sup>2</sup>, *B. de Freminville*<sup>3</sup>, *B. McGillivray*<sup>4</sup>, *D. McFadden*<sup>1</sup>, *V. Adouard*<sup>3</sup>, *D. Terespolsky*<sup>2</sup>, *F. Prieur*<sup>3</sup>, *T. Pantzar*<sup>1</sup>, *M. Hrynychak*<sup>5</sup>. 1) Department of Pathology, UBC, Vancouver, BC, Canada; 2) Department of Laboratory Medicine Credit Valley Hospital Mississauga, ON, Canada; 3) Cytogenetics Laboratory, Jean Monnet University, Saint-Etienne, France; 4) Department of Medical Genetics, UBC, Vancouver, BC, Canada; 5) Cytogenetics Laboratory, Royal Columbian Hospital, New Westminster, BC, Canada.

Prenatally detected chromosome abnormalities are known to have a different prognosis from those detected postnatally because of ascertainment bias of the latter. Postnatally, isodicentric Yp (iso Yp) presents with wide phenotypic variation, from Turner syndrome females to phenotypically normal, infertile males. Few data are available on the outcome of prenatally diagnosed isochromosome Yp. Five cases of prenatally diagnosed iso Yp are presented. Four of the 5 cases had amniocentesis on the basis of advanced maternal age (cases 1-4) and one was diagnosed after nuchal translucency was detected in one twin (case 5). One case showed no other cell lines (case 3) while one had a 45,X cell line and a cell line with two copies of the iso Yp (case 2) and case 4 had a 45,X cell line only. A 45,X cell line alone was present in one case (case 5) and a 45,X cell line and a 46,XY cell line were present in another (case 1). Cases 1-3 resulted in the delivery of normal male infants, with essentially normal follow-up at 3-8 months. Case 4 was terminated and showed a normal male fetus. Case 5, diagnosed after prenatal diagnosis of nuchal translucency, resulted in the birth of a male infant with ambiguous genitalia. These cases of prenatally diagnosed isodicentric Yp demonstrate the cytogenetic variability encountered prenatally and that prenatally diagnosed iso Yp appears to have a good prognosis that results in a phenotypically normal male infant in most cases.

**Terminal 6p deletion detected by subtelomeric screening using fluorescence in situ hybridization.** *C. Le Caignec*<sup>1,2</sup>, *J.M. Rival*<sup>2</sup>, *C. Delnatte*<sup>2</sup>, *M. Boceno*<sup>2</sup>, *A. David*<sup>2</sup>. 1) Fac Medecine, LEPA, Nantes, France; 2) Service de genetique medicale, Nantes, France.

Terminal 6p deletions are relatively rare. However, a distinct clinical phenotype has emerged, including developmental delay, congenital heart malformations, ocular abnormalities, hearing loss, and a characteristic facial appearance. To our knowledge, four patients have been previously reported with detailed FISH analyses. For three of them, the size of the deleted region was estimated to 6 Mb. The fourth case had a smaller deletion, estimated to 2.1 Mb. In contrast to previously reported cases, this patient had no mental retardation, but severe language impairment and difficulties in social interaction. We report a patient with a terminal 6p deletion, detected by subtelomeric screening using fluorescence in situ hybridization (FISH). The girl presented with facial dysmorphic features, hearing impairment, malformation of the anterior eye segment, and mental retardation. Detailed FISH analysis with 17 BAC probes covering the distal 6p25 region estimated the size of the terminal deletion to 6.1 Mb. The breakpoint was located between RP11-232P20 and RP11-525O21 clones. The forkhead transcription factor gene *FKHL7*, involved in a spectrum of anterior eye chamber disorders, is deleted in this patient. These data suggest that patients with complete terminal 6p deletion phenotype, including mental retardation, have a possible major breakpoint at 6 Mb. More patients with detailed FISH analyses are needed to confirm these findings.

**A de novo deletion (5)(q15q22). Further phenotypic description including APC gene analysis. Prognostic and therapeutic implications.** A. Iglesias, B. Davidoff-Feldman. Division of Genetics, Department of Pediatrics, Nassau University Medical Center, East Meadow, NY.

A 5 year-old boy was referred for speech and motor delay. Pregnancy and perinatal period were normal. Birth weight was 3264 gr. At 3 years he was active, partially interactive and barely verbal. Weight, height, and head circumference were normal. He had a narrow forehead with a prominent medial ridge and mild synophris. Eyes were normal. He had a flat nasal bridge and anteverted nares. Philtrum was prominent with full lips. Ears were soft, cupped and rotated. Neck was normal. Chest was flat. Respiratory, cardiovascular and abdominal exams were normal. External male genitalia were normal. Cubitus valgus and joint hyperextensibility were noted. Thenar and hypothenar eminences were flat bilaterally. Neuromuscular exam was essentially normal except for mild strabismus. Skin was normal. Developmental delay was noted. A hearing evaluation, CT scan of the head and cervical spine were normal. Bone age was mildly delay. High-resolution chromosomes showed 46, XY, del (5)(q15q22). Parental chromosomes were normal confirming the de novo nature of the defect. Similar deletions have been linked with multiple congenital anomalies and mental retardation. Moreover a de novo deletion of chromosome 5q21-q22 causing dysmorphic features, mild mental retardation, and Familial Adenomatous Polyposis (FAP) has been reported. Since the deleted region in our patient theoretically encompassed the APC area, cytogenetic analysis was done. Results were normal. Molecular analysis is in progress to rule-out small deletions and/or point mutations in the APC gene before proceeding or not with more invasive procedures (i.e. colonoscopy). Search for APC deletions and mutations in patients with FAP are standard. However, the same is not customary for patients with 5q deletions involving the APC region. Since management and prognosis would dramatically change if the APC gene were not present, detailed analysis of the APC region would benefit these patients. Moreover, comprehensive studies of the area might increase our understanding of the genotype-phenotype correlations in this type of patients.

**Deletion of 9p associated with sex reversal in a patient with multiple congenital anomalies.** *D. Mayen, M. Aguinaga, G. Razo, J.L. Saucedo, R. Garcia-C.* Dept Genetics, Inst Nacional de Perinatologia, Mexico City, Mexico.

**Introduction:** Multiple congenital anomalies have been associated with unbalanced structural chromosomal anomalies. Deletion of 9P has been associated with turricephaly, facial dysmorphism and sex reversal in 70% of the patients. **Case report:** We present a newborn patient who was the first pregnancy of a young couple, the mother had epilepsy of unknown origin treated with valproic acid during pregnancy. The patient had ocular hypertelorism, broad nasal bridge, bilateral cleft lip and palate, small and low-positioned ears, short and broad neck, bivalvular and cardiac insufficiency, bilateral renal cysts, female external genitalia, bilateral camptodactyly in hands, right hand with postaxial polydactyly and anomalous position of fingers of both feet. The karyotype was 46,XY,der(9)t(6;9)(q15;p22), so we performed chromosomal analysis in both parents finding a balanced translocation in the mother: 46,XX,t(6;9)(q15;p22), the father had a normal karyotype. We performed FISH analysis with painting probes for chromosomes 6 and 9 and a probe for the unique sequence SRY. **Conclusion:** We report a patient with multiple congenital anomalies and sexual reversion caused by an unbalanced chromosomal anomaly which deletes autosomal genes involved in sex determination.

**Detection of a cryptic subtelomeric insertion in a child with global development delay.** *F-Y. Han<sup>1,4</sup>, L. Russell<sup>1,2</sup>, C. Morel<sup>1,2</sup>, A. Duncan<sup>1,2,3</sup>*. 1) Department of Human Genetics, McGill University, Montreal, Que. Canada; 2) Department of Pediatrics, Montreal Children's Hospital, Montreal, Que. Canada; 3) Department of Pathology, Montreal Children's Hospital, Montreal, Que. Canada; 4) Laboratory Medicines, Memorial University of Newfoundland, St. John's, NL. Canada.

Cryptic chromosomal rearrangements involving the subtelomeres are emerging as an important cause of human dysmorphism and mental retardation. Clinical investigation of subtelomere rearrangement is now available by FISH analysis using a set of probes specific to the ends of each chromosome. Using FISH with these chromosome-specific subtelomeric probes, we identified a de novo insertion of 9p subtelomeric sequences into the long arm of the X chromosome in a 3 year-old boy with global development delay, hypotonia and esotropia associated with isolated congenital motor nystagmus. Standard cytogenetic assessment of G-banded metaphases at the 500-band level of resolution revealed no structural chromosome anomalies in this patient and his parents. FISH subtelomere analysis with commercial chromoprobe multiprobe-T system revealed an extra subtelomeric signal of 9p on one of the C group chromosomes in all metaphases of the patient. FISH with multiple probes from chromosome 9 (9ptel30) and X (STS and DXZ1) confirmed that the rearranged C group chromosome was the X chromosome. The patient's karyotype was determined to be: 46, XY .ish der (X) ins (X; 9) (q?22; p24p24) (9ptel30+, STS+, DXZ1+). FISH analysis of both parents was negative for subtelomeric rearrangements. Therefore, the subtelomeric insertion detected in the proband is a de novo event. Baker et al (2002) reported a case with a similar rearrangement. However, the X; 9 insertion in that case was paternally inherited and the proband also had a subtelomeric 1pter deletion. Mental retardation and down-slanting palpebral fissures were the only features shared by our patient and the child described by Baker et al. Although cross-hybridization between 9p subtelomeric sequences and Xq has not been reported, we cannot completely eliminate the possibility of a rare interstitial cross-hybridization in our case.

**A new tool for study of multifactorial inheritance: electrocardiographic investigation in Down syndrome families.**

A. Sinkus, L. Jurkeniene, I. Andriuskeviciute. Dept Biol, Lab Cytogenetics, Kaunas Univ Medicine, Kaunas, Lithuania.

There is no one clinical sign which could be typical to chromosome diseases in general or to a single disease. According to our present hypothesis, the chromosome diseases are polymorphic, because the chromosome imbalance decreases the threshold of appearance in familial pathology. In order to detect multifactorial traits with prevalent inherited factors, one must choose i) a sufficiently frequent chromosome disease, and ii) a sufficiently frequent multifactorial sign which could be additionally divided into smaller units. The most frequent of autosomal diseases is Down syndrome (DS). Electrocardiogram (ECG) was used as a typical multifactorial trait. Standard 12-lead ECG was recorded for all of 1004 DS patients and their relatives. The ECGs were studied in a group of 297 DS patients (average age 14.1 years) with cytogenetically proven additional chromosome 21. ECG was taken also for 269 siblings (average age 18.7 years). Should any of the ECG parameters occur with the same frequency in siblings and parents and be present more frequently in DS patients, we might affirm that it reflects hereditary damage of this ontogenetic channel. Therefore ECG was also studied in 438 parents (average age 49.0 years). The formal ECG diagnosis was divided into 21 trait. ECG changes have been found in 77.8% of DS patients, 63.3% of siblings and 69.2% of parents. If trisomy-21 provokes manifestation of multifactorial pathology, one can expect that inheritance of some ECG parameters in chromosome patients would be close to Mendelian. For 10 ECG diagnoses parental trait was inherited by offsprings. Only the difference between the frequencies of inherited bundle branch block in DS patients and their siblings was statistically significant ( $P=0.004$ ). Parents with bundle branch block (right or left, complete or incomplete) had 41 offspring suffering from DS, and for 12 (29.3%) of these bundle branch block was established. In 42 healthy siblings this block was found in three cases (7.1%). The analysis of familial patterns enabled to conclude that the bundle branch block was inherited by DS patients in autosome dominant type with penetrance of 60%.

**ATR-16 due to a chromosomal rearrangement involving partial 4p trisomy.** *L. Titomanlio<sup>1</sup>, D. De Brasi<sup>1</sup>, F. Fabbrini<sup>2</sup>, R. Genesio<sup>2</sup>, A. Conti<sup>2</sup>, L. Pecoraro<sup>1</sup>, M. Tardi<sup>1</sup>, C. Di Stefano<sup>1</sup>, D. Melis<sup>1</sup>, E. Del Giudice<sup>1</sup>, G. Sebastio<sup>1</sup>.* 1) Department of Pediatrics, Federico II University, Naples, Italy; 2) Department of Cellular and Molecular Biology and Pathology, Federico II University, Naples, Italy.

Alpha thalassemia/mental retardation 16 (ATR-16) is a recognizable condition whose constant clinical features are mental retardation (MR) and anemia. Unbalanced chromosomal translocations involving chromosome 16 and resulting in 16p terminal deletions are the most frequent causes of the syndrome. A mild alpha-thalassemic phenotype results from deletion of alpha-globin genes, localized in 16p13.3. We report on a 10-year old girl affected by severe mental retardation, dysmorphic face, and mild microcytic anemia. At birth, intestinal malrotation was diagnosed. Clinical phenotype included: upswept frontal hairline, marked eyebrows with synophris, bilateral epicanthic folds, telechantus, down-slanting palpebral fessures, open held mouth, bilateral simian creases, right talipes, severe mental retardation, mild generalized hypotonia. FISH analysis with telomeric probes disclosed a distal 16p deletion spanning at least 3 Mb, associated with partial trisomy of 4p. Complete karyotype was: 46,XX,der(16)t(4;16)(16q->16p13.2::4p16.2->4pter), paternally derived (father's karyotype: 46,XY,t(4;16)(p16.2;p13.2). The phenotype of the patient overlaps ATR-16 syndrome. The deletion includes alpha-globin genes and, possibly, SOX8 gene, mapping to 16p13.3, whose haploinsufficiency might be related to mental retardation in ATR-16. Trisomy 4p has never been described in association with del 16p. In our patient, it involves approximately 8 Mb of distal 4p. Only some features of the classical trisomy 4p were present. Intestinal malrotation has never been described in ATR-16 and it might represent an accessorial finding of the syndrome. The case here reported outlines the crucial role of the detection of cryptic subtelomeric rearrangements in mentally retarded patients.

**Costello syndrome: report of a child with severe feeding problems.** *S. Ala-Mello*<sup>1,2</sup>. 1) Biomedicum Helsinki, Department of Medical Genetics, Helsinki, Finland; 2) The Family Federation of Finland, Department of Medical Genetics, Helsinki, Finland.

In 1971, Costello described a new syndrome characterised by postnatal growth deficiency with severe failure to thrive and feeding problems, coarse facial features, loose soft skin with deep palmar and plantar creases, benign nasal papillomata, hypertrophic cardiomyopathy and developmental delay. During the last ten years this multiple congenital anomaly syndrome has been increasingly recognized. The pathogenesis and molecular basis of the syndrome are unknown and the diagnosis is based on the characteristic pattern of anomalies. This is a report of a child with Costello syndrome having extremely severe feeding problems. This girl is the first child of healthy unrelated parents. Birth weight in the 36th gestational week was 3950 g and length 48 cm. Since the birth she has suffered from vomiting and feeding problems. She was treated with naso-gastric tube. The feeding problems continued and one year ago at the age of one year and nine months gastrostoma was operated. This has not improved the growth. Since the birth she has been sweating a lot, but the reason for that has been unknown. The skin of the palms and feet is loose. The characteristic facial features are described and shown by the photographs. At the age of nine months she was operated because of hydrocephalus. Hypertrophic cardiomyopathy was also found, and has been later followed up by cardiac ultrasound. Now, at the age of three years she still has gastrostoma.

**Egyptian patient with OFD I&II Phenotypes overlap:brief clinical report.** *M.M. Abuhenedi, R.L. Alnaggar, S. Alawadi, L. Bastaki.* Obstetric & Gynecology, Kuwait Medical Genetics Center, Kuwait, Kuwait.

We describe a female patient aged 6 months-old. She was a product of preterm/ND after uneventful pregnancy. Her parents were blood relatives and there were no similar conditions in the family. She had feeding difficulties and failing to thrive. The DQ assessment was below 3rd percentile. Somatic features included facial dysmorphism, overhanging forehead, hypertelorism, proptotic eyes, broad nasal root, low set/posteriorly rotated ears, micrognathia, high/narrow arched palate and hyperplastic lingual tissue. Limb anomalies included clinodactyly, unilateral polysyndactyly of the big toe have been found, as well as congenital hip dislocation, VSD and conductive deafness. Skeletal survey confirmed limb anomalies and chromosomal analysis showed normal female karyotype. The girl had an overlapping OFDI/OFDII phenotype. The mode of inheritance could be autosomal recessive.

**Congenital anterior cervical hypertrichosis and mental retardation: a new association?** *J.R. Corona-Rivera<sup>1,2</sup>, A. Corona-Rivera<sup>1,3</sup>, L. Bobadilla-Morales<sup>1</sup>, S. González-Abarca<sup>2</sup>, J. Hernández-Rocha<sup>2</sup>, D. García-Cruz<sup>4</sup>*. 1) Laboratorio de Genética Humana, C.U.C.S., Universidad de Guadalajara, Guadalajara, Jalisco, México; 2) División de Pediatría, Hospital Civil de Guadalajara Dr. Juan I. Menchaca, Guadalajara, México; 3) Unidad de Citogenética, Hospital Civil Fray Antonio Alcalde, Guadalajara, México; 4) División de Genética, Centro de Investigaciones Biomédicas de Occidente, IMSS, Guadalajara, México.

Congenital anterior cervical hypertrichosis (CACH) refers to the presence of a patch of hypertrichosis just above the laryngeal prominence. To the best of our knowledge, CACH have been reported in 9 previous cases. We report a boy with CACH associated with mental retardation. We review the phenotypic variation of sporadic and familial reports of CACH and propose a new association. The proband was a product of normal gestation and delivery except for hemorrhage during the third trimester. BW was 2800 g. He showed developmental delay and null scholastic achievements. Physical examination at 11-years-old showed weight 27.5 kg (-1.74 SD), height 128 cm (-2.08 SD), OFC 48 cm (-3.5 SD), synophris, fine short vellus blonde hairs on the face, CACH, and excessive hair growth on back. Inverted nipples, clinodactyly of the fifth right finger, hallux valgus and left overriding third to second finger toes were also observed. Fundoscopic examination of the eye was normal. Psychometric evaluation achieved an IQ score of 39 on the WISC-R test (verbal IQ was 46, and performance IQ score was 41), corresponding to moderate mental retardation. Intracranial structures were normal at cranial CT scan. EEG was abnormal. Nerve conduction studies, electromyography, T3, T4, TSH, and metabolic screening test were normal. Karyotype was 46,XY. CACH as isolated trait is a rare form of localized hypertrichosis with suggested dominant inheritance, either X-linked or autosomal (OMIM 600457). CACH has been associated with hereditary motor and sensory neuropathy (HMSN), severe chorioretinal pathology and optic atrophy with probably autosomal recessive inheritance (OMIM 239840). This report supports the existence of a new association of CACH-mental retardation without HMSN.

**Mandibuloacral dysplasia due to homozygosity for the R527H mutation in lamin A/C.** *J.J. Shen<sup>1</sup>, C.A. Brown<sup>3</sup>, J.R. Lupski<sup>1, 2</sup>, L. Potocki<sup>1</sup>.* 1) Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept of Pediatrics, Texas Childrens Hospital, Houston, TX; 3) Molecular Diagnostics Laboratory, Fairview-University Medical Center, Univ of Minnesota, Minneapolis, MN.

Mandibuloacral dysplasia (MAD) is a rare autosomal recessive disorder characterized by mandibular hypoplasia, acro-osteolysis, joint contractures, poikiloderma, and lipodystrophy. Recently, a specific point mutation (R527H) in *LMNA*, which encodes lamins A and C, was reported to cause MAD.

We present the clinical features of a 12-year-old Latin American male with MAD. This patient was phenotypically normal until approximately 1 1/2 years of age and progressively developed camptodactyly, limb contractures, lipodystrophy, regional skin atrophy and poikiloderma, unusual facies, micrognathia, and dental crowding. He has normal cognitive function and has no history of chronic illnesses. Skeletal survey revealed generalized osteopenia and acro-osteolysis. Sequence analysis of *LMNA* revealed the R527H mutation, yet the haplotype in our patient is distinct from that of the previously reported families.

Lamins are important structural components of the nuclear lamina with hypothesized roles in many different cellular processes. In addition to MAD, other diverse diseases caused by mutations in *LMNA* and other lamins (collectively referred to as the laminopathies) include Charcot-Marie-Tooth type 2B, dilated cardiomyopathy, Emery-Dreifuss muscular dystrophy, familial lipodystrophy, limb-girdle muscular dystrophy, and Hutchinson-Gilford progeria. For most of these laminopathies, allelic heterogeneity has been reported. In contrast, the vast majority of Hutchinson-Gilford progeria cases result from a single mutation. In families with MAD in which a mutation has been described, a similar pattern is emerging as all are homozygous for the identical R527H substitution in *LMNA*. Interestingly, haplotype analysis of affected individuals reveals that in each case, the mutation arose independently. Thus, a highly specific genotype-phenotype correlation appears to exist for the *LMNA* R527H mutation and MAD.

**3D dense surface modeling defines a characteristic facial phenotype in Bardet-Biedl syndrome.** *P. Hammond<sup>1</sup>, N. Hindocha<sup>2</sup>, T. Hutton<sup>1</sup>, P.L. Beales<sup>2</sup>.* 1) Biomedical Informatics, Eastman Dental Institute, London, UK; 2) Molecular Medicine Unit, Institute of Child Health, London, UK.

For the Bardet-Biedl syndrome (BBS), a genetically heterogeneous disorder characterised by retinal dystrophy, obesity, polydactyly, renal and cognitive impairment, indistinct facial features have been reported. We sought to determine a reproducible BBS facial phenotype objectively through novel 3D dense surface modelling of the face. We acquired 3D face images of 29 UK BBS patients. 21 were matched for age (8 - 50 yrs) and race to 83 Caucasian controls. A dense surface model, principal component analysis (PCA) of the densely corresponded face surfaces, was computed. Using in-house software we sought key differences for the two groups in 1) 3D morphs of mean faces 2) mode distributions 3) virtual anthropometric measurements. Portrait and profile views of morphs between the mean BBS and control faces strongly suggest a relatively shorter, narrower nose; a broader zygomatic arch; and a longer lower-face. The ears are also set lower than the controls. These are confirmed by virtual measurements. There is significant bias for the BBS group in the distribution of two higher modes, one emphasising the mid-face findings above and the other retrognathic posture. As BBS is genetically heterogeneous we also focussed on those patients who were homozygous for the same BBS1 mutation, M390R, the result of a single base substitution (n=9). Even more marked differences in the morphs and modes were noted suggesting this is a consistent and reproducible phenotypic trait with a common underlying molecular pathology. The overall impression is that of midface retraction giving the appearance of a dishd face with the net effect that the nose is turned up. This novel method of defining subtle dysmorphic features has confirmed structural anomalies of the midface consistent with the same underlying gene mutation. This is not surprising as high-arched palate, dental anomalies and phonation defects have been reported in a majority of BBS patients. These findings should aid clinicians in the diagnosis of BBS and provide clues to the developmental role of the underlying genes.

**The prevalence of major congenital malformations in AL-Jahra regional liaison community of Kuwait.** *R.L. Alnaggar<sup>1</sup>, S.A. Madi<sup>2</sup>, S.A. AL-Awadi<sup>1</sup>, M.M. Abu-Henedi<sup>1</sup>, M.J. Marafie<sup>1</sup>, R. Mary<sup>1</sup>, M. Krishna<sup>1</sup>, L. Bastaki<sup>1</sup>.* 1) Dept Genetics/OB, Kuwait Medical Genetic Ctr, Nasser, Kuwait; 2) Pediatric Department AL-Jahra Hospital.

Eighty percent of AL-Jahra region population is of Bedouin origin and they have high rate of consanguinity. The major congenital malformations(MCM) have high incidences among the nations of Arabian Gulf. We aim to establish a profile of these malformations in this area of Kuwait. 7739 newborn babies were ascertained by family history,clinical examinations and the relevant investigations were done. 97 babies were found to have MCM with incidence 12.5/1000 births.49 babies(50.6%)had multiple system anomalies and 48 babies(49.4%) had single system anomaly.21 babies (42.8%) had recognized syndrome,most of which were AR disorders.17 babies had chromosomal anomalies.Isolated system anomalies included CNS(12 cases),CVS(9 cases),Skeletal system(7 cases)and GIT(6 cases).68% of couples were consanguinous compared to 56% in controls( $P<0.01$ ).The study showed the high prevalence of MCA among Bedouins despite of many cases escaped diagnosis and registration.The genetics services should provided as an effective means of preventions.

**Genetic epidemiology of the exstrophy-epispadias complex.** *S.A. Boyadjiev Boyd<sup>1</sup>, J.L. Dodson<sup>2</sup>, C.L. Radford<sup>1</sup>, T.H. Beaty<sup>3</sup>, R.I. Mathews<sup>2</sup>, J.P. Gearhart<sup>2</sup>.* 1) McKusick-Nathans Inst Genetic Medicine, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Divison of Ped Urology, The James Buchanan Brady Urological Institute, Johns Hopkins Univ Sch Med; 3) Dept of Epidemiology, Johns Hopkins University.

Exstrophy-epispadias complex (EEC) is a spectrum of midline urologic defects ranging in severity from epispadias (EP) and classic bladder exstrophy (BE), to cloacal exstrophy (CE). **Hypothesis:** EEC is a complex trait caused by both genetic and non-genetic factors. **Methods and results:** Through our pediatric urology clinic and an advertisement on the Internet, we have identified 204 EEC families, 5 of which (2.5%) were multiplex. Proband in these families were 95% Caucasians. Male predominance was significant in the EP group (3.2:1; n=25), less evident in the BE group (1.6:1; n=151) and absent in the CE group (1:1; n=20). No significant differences were observed in the analyses of paternal age, birth weight, reproductive history of the families, and exposures during the first trimester. The median maternal age at birth was 29 (n=103), slightly higher than national averages corresponding to birth year. More than half (54%) of the probands were first born. Two patients with BE had omphalocele and one had spina bifida, suggesting intermediate phenotypes between BE and CE. Five probands had renal defects and 7 had anomalies outside the EEC spectrum. Twenty-two probands (23.6%) had relatives with congenital anomalies. Of interest, cleft lip and palate (CLP) was observed in 7 families and midline defects (omphalocele, imperforated anus, spinal defects, hypospadias) were present in 11 families. 112 families donated biologic specimens for molecular studies. Karyotype analysis on 39 cases detected two chromosomal abnormalities- 46, XY, t(8;9)(p11.2;q13) and 47, XYY. **Conclusions:** The observed differences in the sex ratio suggest a stronger genetic component for CE than for EP or BE. Further studies are needed to evaluate race, advanced maternal age, and birth order as possible risk factors for EEC. Unexpected aggregation of birth defects in EEC families, specifically CLP and midline defects, warrant detailed clinical genetic evaluation of all families and may help identify putative candidate genes.

**Variable expression of abdominal wall defects and external genital anomalies in a three generation family.** S. Fokstuen, A. Wonkam. Division of Medical Genetics, Geneva, Switzerland.

**Background:** A few familial cases of isolated abdominal wall defects with variable expressivity have previously been reported. We report a three generation family with either variable abdominal wall defects or external genital anomalies as isolated defects suggesting a possible common genetic background of the two groups of congenital malformations.

**Case :** A 27 year old man was referred for genetic counselling following the birth of a female child with isolated gastroschisis. Family history revealed that he had with another woman a daughter with hypoplasia of the external genitalia and synechia of the labia minora. His father was born with an umbilical hernia and the consultant himself, who has no brothers and sisters suffered from umbilical hernia with associated hernia of the linea alba.

**Conclusion :** This pedigree supports a vertical mode of transmission of abdominal wall defects carried through the father as well as the hypothesis that in some families gastroschisis and umbilical hernia are different expression of the same primary defect. In addition our family suggests that embryologically external genital anomalies may represent an additional malformation within the spectrum of abdominal wall defects. This observation may be considered in genetic counselling of families with recurrence of abdominal wall defects.

**Autosomal dominant nonsyndromic cleft lip and palate linked to chromosome 4.** *M. Edwards*<sup>1, 2</sup>, *L. Roddick*<sup>2, 3</sup>, *R. Scott*<sup>2, 4</sup>. 1) Hunter Genetics, Hunter Area Health Service, Waratah, New South Wales, Australia; 2) Faculty of Health, University of Newcastle, NSW Australia; 3) Department of Paediatrics, John Hunter Hospital, Newcastle, NSW; 4) Department of Genetics, Hunter Area Pathology Service, Newcastle, NSW.

There were seven affected relatives in six generations of a family with autosomal dominant non-syndromic cleft lip and palate, with no consanguinity. Fourteen unaffected family members, three affected relatives and three obligate carriers who were non-penetrant were assessed by a clinical geneticist. None had oligodontia, nail abnormalities or any other syndrome associated with orofacial clefts. Nineteen relatives from four generations contributed blood samples for linkage study. Cleft lip with or without cleft palate has been mapped to chromosomes 2, 4, 6, 14, 17 and 19 in European populations and chromosomes 1, 2, 3, 4, 6, 18 and 21 in Chinese families (Carinci et al 2000 *Cleft Palate Craniofac J* 37:33-40; Prescott NJ et al *Hum Genet* 106:345-50; Marazita et al 2002 *Am J Hum Genet* 71:349-364). Mutations in *MSX1* have recently been identified in families with Witkop tooth-nail syndrome, in families with cleft lip or palate and oligodontia, and most recently in families with non-syndromic cleft lip or palate (Jesewski PA et al 2003, *J Med Genet* 40:399-407). Markers from these regions were analyzed in the family. The results revealed that there was positive linkage to two chromosomal loci, one on 4p in the vicinity of the *MSX1* gene (D4S3023) with a score of 0.40 and a second locus on 4q, which revealed a much stronger association with a maximum score of 1.66 (D4S402). The linkage results, although not highly significant point to the possibility that another locus exists at position 4q26. Further analysis will be undertaken to identify the genetic basis of this disease in this family.

**Bartsocas-Papas syndrome further Mediterranean cases.** *F.S. Al-Harbi<sup>1</sup>, E.A. Al-Awad<sup>2</sup>, W.E. Eyaid<sup>3</sup>.* 1) pediatrics, NorthWest armed Forces Hospita, Tabook, Northen region, Saudi Arabia; 2) Pediatrics, NorthWest Armed Forces Hospital. Tabook 2424, Northen Region. Saudi Arabia Essa H. Al-Awad pediatrics, NorthWest armed Forces Hospita, Tabook, Northen region, Saudi Arabia; 3) Wafaa Eyaid, Pediatrics, King Fahad Hospital, Riyadh, Central region. 11246.p.o box 22490.

Bartsocas-Papas syndrome, BPS (omim 263650) is an autosomal recessive disorder characterized by oral cleft, filiform bands between the jaws, ankyloblepharon, popliteal pterygium, syndactyly of fingers and toes, phalangeal anomalies with synostosis, club feet, nail hypoplasia, and genital anomalies. Additional traits included cutis aplasia, widely spaced nipples, low-set umbilicus, and unilateral renal hypoplasia. We report on 5 infants who had BPS. The index case was a 4-days old baby who was born at 35 weeks by breech with birth weight of 2.3 kg. He was microcephalic, bald, with wide anterior fontanel, absent nose bridge, anophthalmia, severe bilateral cleft, lip and palate, forehead scars, absent hands, ambiguous genitalia, no palpable gonads. There was a tail-like band at the pubic area, and another band at the anus extending posteriorly. Parents are far relatives. One brother died at the age of 11-months with similar congenital anomalies. Also, there were 3 maternal first cousins who died in early infancy period with the same congenital anomalies. The parents were first cousin. This is the 8th family from the Mediterranean area reported with this very rare condition. Banking of DNA on these cases is important for further study of these cases.

**Lateral Meningocele Syndrome: Vertical Transmission and Expansion of the Phenotype.** *K. Chen<sup>1</sup>, L.M. Bird<sup>2</sup>, P. Barnes<sup>1</sup>, R. Barth<sup>1</sup>, L. Hudgins<sup>1</sup>.* 1) Stanford University, Stanford, CA; 2) Children's Hospital and Health Center and University of California, San Diego, CA.

Lateral meningoceles were first described by Lehman et al. in 1977 in a patient with other skeletal findings and distinctive craniofacial features. Subsequently, six more patients with the so-called lateral meningocele syndrome (LMS) have been reported. We describe the findings in three new patients with this condition, which expand the phenotype and support the hypothesis that this is an autosomal dominant disorder affecting primarily the connective tissue.

In addition to multiple arachnoid cysts of the spinal canal, Case 1 was noted to have hypertelorism, down-slanting palpebral fissures, ptosis, low-set ears, and micrognathia. Case 2, the mother of case 1, exhibited similar craniofacial features as well as palpable bilateral flank masses, mild scoliosis, hearing loss and a congenital umbilical hernia. Both individuals exhibit normal intelligence, short stature and significant joint hypermobility. History of hypotonia is denied in these two cases. Case 3 exhibited cleft palate, VSD, inguinal hernias, and hypotonia. If one combines the findings in our cases with those found in the six previously reported cases, it is clear that there is a consistent craniofacial phenotype comprised of ocular hypertelorism (5/7), downslanting palpebral fissures (9/9), ptosis (7/7), low-set posteriorly rotated ears (8/8), high arched palate (9/9) and micrognathia (9/9). We were also impressed with the frequency of findings suggestive of an underlying connective tissue disorder including joint hypermobility (5/7), scoliosis/kyphosis (7/9), umbilical/inguinal hernias (4/5), and Wormian bones (4/6). Procollagen studies in one previously reported case were normal.

The findings present in the mother and child support an autosomal dominant pattern of inheritance for LMS. The connective tissue findings in our three patients and the previously reported patients suggest that an underlying defect of the connective tissue is responsible for this condition.

**A case of acrocallosal syndrome complicated with congenital nephrotic syndrome.** *K. Nakashima<sup>1</sup>, T. Sato<sup>1</sup>, T. Hasuwa<sup>1</sup>, T. Kondoh<sup>1</sup>, T. Taguchi<sup>2</sup>, M. Noguchi<sup>3</sup>, M. Aoki<sup>4</sup>, H. Moriuchi<sup>1</sup>.* 1) Department of Pediatrics, Nagasaki University School of Medicine, Nagasaki, Japan; 2) Department of The Second Pathology, Nagasaki University School of Medicine, Nagasaki, Japan; 3) Department of Urology Nagasaki University School of Medicine, Nagasaki, Japan; 4) Department of Pediatrics, National Nagasaki Medical Center, Nagasaki, Japan.

Acrocallosal syndrome (ACS) is a congenital malformation disorder characterized by hypoplasia/aplasia of corpus callosum, polydactyly, craniofacial anomalies, and severe psychomotor retardation. We here report an ACS patient complicated with congenital nephrotic syndrome (CNS). Patient, an 8-month-old Japanese boy, is the first child of non-consanguineous healthy parents. Family history was unremarkable. He was born at 40 gestational weeks as AFD. Clinical manifestations included wide fontanelles, prominent forehead, telecanthus, micrognathia, low-set ears, stenosis of both external ear canals, bilateral broad thumbs, ectopic unilateral testis, and postaxial polydactylies of the hands and feet. Systemic bone surveys showed the 13th ribs. UCG showed mild myocardial hypertrophy. Proteinuria was noted soon after birth. His developmental milestones were delayed. Neuroimaging studies showed thin corpus callosum and fluid retention of bilateral internal ears. ABR revealed bilateral mixed-type hearing loss. Ophthalmological examination showed bilateral macular degenerations. His karyotype was normal, 46,XY. Because of CNS, he underwent kidney biopsy at 8 months of age, which was compatible to CNS. Four-weeks treatment with prednisolone was ineffective. CNS is a novel complication of ACS. The etiology of ACS is unknown. Pfeiffer et al. (1992) suggested that the mutation for ACS located on 12p from his patient with a de novo inverted tandem duplication of 12p13.3-p11.2. High-resolution chromosomal examination for his chromosome 12 failed to identify any mutation, however. As regards CNS, two genes, nephrin located on chromosome 19q13.1 and podocin on 1q25-q31, are identified as causative genes. Thus, our case may suggest that the gene locus of ACS locates near 19q13.1 or 1q25-q31, or one gene for the CNS locates near the gene locus for ACS.

**Nonsyndromic Supernumerary Teeth in Families.** *E. Severin*<sup>1</sup>, *D. Stanciu*<sup>2</sup>. 1) Department of Human Genetics, "Carol Davila" University of Medicine and Pharmacy, Bucharest, ROMANIA; 2) Department of Orthodontics, "Carol Davila" University of Medicine and Pharmacy, Bucharest, ROMANIA.

Supernumerary teeth in the permanent dentition is relatively common in man. Heredity is often involved, but specific genes are not identified. In dental practice occurs a vast spectrum of clinical manifestations in patients with extra teeth. Objective: the aim of our study was to clarify the relationship between variation in clinical phenotypes and genetic heterogeneity. Subjects and methods: the study population comprised 13 patients with supernumerary teeth, aged 10-27 years, and their family members. The diagnosis of supernumerary teeth was based on both clinical and radiographic examinations. Pedigrees were constructed according to data from family history. Segregation analysis was used to determine the mode of inheritance. Statistical analysis was carried out too. Results: phenotypic variation included the number, location and morphologically type of the supernumerary teeth. 92.3% of the probands had one isolated extra tooth and 7.7% had multiple supernumerary teeth. The most common region of the jaws affected was premaxilla. The most frequent permanent supernumerary tooth was mesiodens, conically shaped, followed by the supplemental upper lateral incisor and paramolar. Extra teeth were erupted or impacted. Associated tooth number abnormalities were recorded. Familial inheritance occurred and involved two or three generations. Pedigrees of nine probands indicated an autosomal dominant mode of transmission with complete penetrance and variable expressivity. Sex-linked inheritance pattern was observed in one family. In three families the dental anomaly did not follow a simple mendelian pattern, but showed a familial predisposition to increase the number of teeth. Familial multiple supernumerary teeth showed a complex inheritance. Conclusions: supernumerary teeth are genetically heterogeneous. Various clinical phenotypes of an isolated extra tooth are determined by mutations in different genes (locus heterogeneity). Familial multiple supernumerary teeth, which are not part of a syndrome, involves an interaction between polygenic and environmental factors within initiation stage of tooth morphogenesis.

**Anophthalmos with limb anomalies: Three cases in a Japanese family.** *T. Tohma<sup>1</sup>, K. Ameku<sup>1</sup>, M. Miyagi<sup>1</sup>, S. Shimabukuro<sup>1</sup>, H. Ageda<sup>1</sup>, Y. Chinen<sup>2</sup>, Y. Izumikawa<sup>3</sup>, K. Naritomi<sup>4</sup>.* 1) Dept Pediatrics, Okinawa Prefectural Naha Hosp, Okinawa, Japan; 2) Dept Pediatrics, Univ Ryukyus Sch Medicine, Okinawa, Japan; 3) Nago Ryoikuen , Okinawa, Japan; 4) Dept Medical Genetics, Univ Ryukyus Sch Medicine , Okinawa, Japan.

Anophthalmos with limb anomalies is a rare autosomal recessive disorder characterized by eye malformations and acromelic malformations. There are many reported cases from Turkey , and most of them are from consanguineous parents. We report on two brothers and a sister from a nonconsanguineous Japanese family, who have bilateral microphthalmia and oligodactyly of toes. The elder brother was stillbirth. He also had holoprosencephaly as an additional finding. The younger brother had an operation for right cryptorchidism. This is the first report from Japan.

**Tetrasomy 9p with no apparent phenotypic characteristics.** *S.N.J. Sait<sup>1</sup>, M. Wetzler<sup>2</sup>*. 1) Clinical Cytogenetics Lab, Roswell Park Cancer Inst, Buffalo, NY; 2) Department of Medicine, Roswell Park Cancer Inst, Buffalo, NY.

Tetrasomy 9p is a rare syndrome described in about 30 cases in the literature. Cases with tetrasomy 9p appear to have characteristic phenotypic features which include facial dysmorphism, developmental delay, central nervous system and limb defects, heart and renal anomalies and the presence of abnormal genitalia. Survival in patients with this defect varies from a few hours to several years, however, the effects of the anomaly are fairly severe. We present a new case of tetrasomy of 9p in a 41-year old male with no apparent phenotypic malformations who presented with complaints of skin lesions. Blood work revealed hypereosinophilia which was confirmed by a bone marrow aspiration and biopsy. Karyotypic analysis of the bone marrow revealed tetrasomy 9p in all cells analyzed. This was confirmed by fluorescence in situ hybridization (FISH) using the LSI p16(9p21)/ CEP 9 probe (Vysis; Inc). Subsequent analysis using PHA stimulated lymphocytes revealed the presence of the tetrasomy in 43% of cells analyzed. A comparison of the results using the FISH probe showed the anomaly to be present in 86% of bone marrow cells while in the PHA stimulated lymphocytes it only occurred in 43% of cells. Review of the literature shows that the degree of mosaicism appears to correlate with the extent of the phenotype and particularly with the severity of prognosis. Over 60% of nonmosaic patients die in the first couple of months after birth. The patient presented, however, showed no evidence of any phenotypic anomalies apart from hypereosinophilia.

**Conductive deafness, choanal atresia and Treacher Collins-like facial dysmorphia : Burn syndrome or autosomal recessive TCS.** *L. Van Maldergem<sup>1</sup>, A. Splendore<sup>2</sup>, M.R. Passos-Bueno<sup>2</sup>, J. Desir<sup>1</sup>, Y. Gillerot<sup>1</sup>*. 1) Centre de Genetique Humaine, Institut de Pathologie et de Genetique, Loverval, Belgium; 2) Department of Biology, Instituto de Biociencias, Universidade de Sao Paulo, Sao Paulo, Brazil.

A 10 y-old girl born to Turkish first cousins was referred for a dysmorphologic advice during her evaluation for a syndromic conductive deafness. A malformation of uncus with fusion of the ossicles, for which she underwent a curative surgery (she recovered hearing) six months earlier, was associated with malar hypoplasia and microretrognathism. A diagnosis of probable recessive Treacher Collins syndrome was made. A search for a mutation in the TCOF1 gene encoding treacle protein was negative. After the birth of a normal sister, a severely affected male was delivered. Marked downward slant of palpebral fissures, malar hypoplasia and the same conductive deafness due to ossicles malformation was present. A first cousin (also born to consanguineous parents) presented at birth with choanal atresia and microretrognathism but no conductive deafness. Although the clinical picture is similar in many respects to the so-called autosomal recessive Treacher Collins syndrome, of which at least ten pedigrees have been reported in medical literature, it is also compatible with a new entity described by Burn in 1992. The clinical picture consists of a TCS-like facial dysmorphia, choanal atresia, conductive deafness, congenital heart disease and autosomal recessive inheritance. In the present family, a homozygosity mapping study is likely to be successful due to its large size and to multiple inbreeding.

**Hypohydrotic ectodermal dysplasia with immune deficiency in a family with a mutation in the NEMO gene.** *J.J. van den Ende, N. Peeters, K. Storm.* Medical Genetics, University of Antwerp, Antwerp, Belgium.

Hypohydrotic ectodermal dysplasia (HED) is a congenital disorder of teeth, hair and eccrine sweat glands, that is mostly inherited in an X-linked way. The combination of X-linked HED with immunodeficiency (HED-ID) causes severe infections in males, with high morbidity and mortality, whereas in females a very variable picture is seen, with hypodontia or conical teeth, and skin abnormalities with spotty hyperpigmentation as in Incontinentia Pigmenti (IP), but no vesicular or verrucous stage as in the classical IP. Classical IP is caused by mutations in the IKK-gamma (NEMO) gene, which activates transcription factor NF- $\kappa$ B, necessary for activation of multiple target genes involved in normal T and B cell development. In 80% of the classical IP cases a recurrent genomic deletion of exons 4-10 of the NEMO gene is found, leading to a complete loss of IKK-gamma function. In HED-ID `hypomorphic` (less severe) mutations are found in the C-terminal region of the NEMO gene, that diminish, but do not eliminate stimulation of NF- $\kappa$ B, with preservation of partial IKK-gamma function. This does not lead to lethality in utero in males, showing normal numbers of B and T cells, but gives dysgammaglobulinemia and defective ability to produce some antibodies. Because of the ectodermal abnormalities the IKK protein might also be involved in development of teeth and eccrine sweat glands. We describe two sisters who showed abnormal pigmentation over the entire body, following the Blaschko lines, from birth on. They never showed vesicles or a verrucous stage. Their mother showed the same pigmentation disorder, as well as a third sister, who died at the age of 1 year in an accident. A younger brother didn't show the abnormal pigmentation, but died at the age of 2 months of meningitis. Both sisters showed some hypoplastic teeth. Because of the unusual pattern and absence of the vesicular and verrucous stage classical IP was less likely. Since the brother died of an infection HED-ID was a possibility and mutation detection of the C-terminal region of the NEMO gene was performed. Indeed a mutation (1167ins C in exon 10) was found, confirming the diagnosis.

**Evidence of unique dermatoglyphic phenotypes in Nail Patella Syndrome (NPS).** *C.A. Brandon<sup>1</sup>, N.M. Scott<sup>1</sup>, K. Neiswanger<sup>1</sup>, S.M. Weinberg<sup>1</sup>, A.L. Towers<sup>2</sup>, M.L. Marazita<sup>1</sup>.* 1) Center for Craniofacial and Dental Genetics, Sch of Dental Med, Univ of Pittsburgh; 2) Div Geriatric Med, Univ of Pittsburgh.

NPS is a rare genetic disease (22/1,000,000) due to mutations in the LMX1B gene, which has been shown to play a major role in dorsoventral patterning and limb development. NPS often affects the distal limb; clinical features include nail dysplasia, triangular lunulae, and loss of skin creases over the distal interphalangeal joints. Abnormal dermatoglyphics have been reported in a number of genetic syndromes affecting the distal limb, however the dermatoglyphics of NPS have never been characterized. Therefore, we investigated dermatoglyphic data from 29 NPS cases, ascertained at the Fifth International Symposium on Nail Patella Syndrome (sponsored by Nail Patella Syndrome Worldwide). Pattern types analyzed by three independent raters and were categorized into ulnar loop, radial loop, whorl, or arch. Approximately 3% of the fingerprint patterns observed in the NPS cases were unique and therefore categorized as other. Control pattern data was obtained from the literature (n= 720). The frequency of arches was significantly increased in affected individuals compared to controls (16.1% vs 4.7 %,  $p < 0.0001$ ) while ulnar loops and radial loops were significantly decreased ( $p=0.01$  and  $p=0.01$ , respectively). Affected males had more whorls ( $p=0.006$ ) and arches ( $p < 0.001$ ), and fewer ulnar loops ( $p < 0.0001$ ) than controls. Affected females also had more arches ( $p < 0.0001$ ), and fewer ulnar loops ( $p < 0.0001$ ) than controls, but fewer whorls ( $p=0.01$ ). Interestingly, as is seen in the general population, females with NPS have more ulnar loops than males ( $p < 0.0001$ ), and males have more whorls than females ( $p < 0.0001$ ). This is the first study of dermatoglyphic patterns in NPS. Our results clearly demonstrate the impact of the LMX1B gene on the dermatoglyphic phenotype of individuals with NPS, specifically resulting in an increase in arches, ulnar loops and unique patterns. Supported by NIH grant DE-13076.

**A case of Restrictive Dermopathy with survival beyond the neonatal period and novel experience with thalidomide therapy.** *P. Pan*<sup>1</sup>, *K. Omlin*<sup>2</sup>, *J. Rosenthal*<sup>2</sup>, *O. Padilla*<sup>3</sup>, *M. Stadecker*<sup>3</sup>, *L. Demmer*<sup>1</sup>. 1) Dept Pediatrics, Division of Genetics, NEMC Box 394, Tufts-New England Medical Ctr, Boston, MA; 2) Dept Dermatology; 3) Dept Pathology.

We report a case of a now 9 m/o male with Restrictive Dermopathy (RD) who was born 2.4kg at 37wk gest. to unrelated parents. Fetal movements were reportedly normal. SROM occurred 10 days PTD. Micrognathia and contractures of knees/ankles and thickened abdominal skin were noted at birth. Karyotype was 46XY. He presented at 7 wks w/FTT and constant vomiting. The skin over his chest, abdomen, back, buttocks and extremities was now thick, tight and bound-down; the skin of the head was relatively spared but scalp veins were very prominent. Joint contractures had worsened. Skin biopsy was characteristic for RD showing thickened epidermis with absent rete ridges, collagen fibers arranged parallel to the epidermis, decreased elastic fibers, increased connective tissue mucin at the dermo-pannicular junction and poorly developed skin appendages. Skin restriction, poor weight gain despite continous NG feeds, and respiratory difficulty progressed over the next six weeks. Thalidomide was begun at 3.5 mo. of age. Repeat biopsy after 4 months of treatment showed no significant change however clinically the skin has softened and the tachypnea and contractures have improved.

RD, usually lethal in the neonatal period, is an AR genodermatosis characterized by prematurity, thickened skin which restricts chest wall and abdominal movement, contractures, and typical facies and skin biopsy findings. Differential diagnosis includes progeria and Parana Hard Skin Syndrome. The etiology of RD is unknown; hypotheses include a defect in collagen synthesis or orientation, inflammatory-mediated disease and modified epidermal-dermal differentiation. This case is unusual in that, due to the later onset of skin restriction, pulmonary hypoplasia was absent at birth. The apparent arrest in progression of symptoms may indicate a response to the immunomodulatory effects of thalidomide, or may represent the natural course of this milder presentation of RD, allowing for survival beyond the neonatal period.

**A novel elastin gene mutation results in autosomal dominant form of cutis laxa.** *L. Rodriguez-Revenga*<sup>1,3</sup>, *P. Iranzo*<sup>2</sup>, *C. Badenas*<sup>1,3</sup>, *S. Puig*<sup>2,3</sup>, *A. Carrio*<sup>1,3</sup>, *M. Mila*<sup>1,3</sup>. 1) Genetics Service, Hospital Clinic, Barcelona, Barcelona, Spain; 2) Dermatology Service. Hospital Clinic. Barcelona. Spain; 3) IDIBAPS (Institut d'Investigacions Biomediques Agusti Pi i Sunyer). Hospital Clinic. Barcelona. Spain.

Cutis laxa is an extremely rare disorder characterized by marked skin laxity. Less than a few hundred cases worldwide have been described. Clinical presentation and mode of inheritance show considerable heterogeneity. Autosomal dominant, autosomal recessive, and X-linked recessive patterns have been noted in inherited forms. Only three mutations in the elastin gene have been reported as the genetic cause of the autosomal dominant form of cutis laxa. A 45 year-old woman and her 19 year-old son were clinically diagnosed of cutis laxa as their skin was inelastic, loose-hanging and wrinkled, with premature ageing appearance. Mutational analysis of the elastin gene evidenced a novel mutation (2292delC) that predicts a frameshift in the coding region and causes translation to proceed into the 3-untranslated region. This would replace the C-terminal amino acid of the normal elastin protein with a novel sequence. This is one of the few cases described in the literature presenting autosomal dominant cutis laxa in which a mutation in the elastin gene has been detected. Acknowledgements: Marato-TV3 98TV1110.

**Dermatoglyphics in Filipinos with nonsyndromic cleft lip with or without cleft palate (NS CL/P), and their relatives.** *N.M. Scott<sup>1</sup>, S.M. Weinberg<sup>1</sup>, S. Daack-Hirsch<sup>2</sup>, K. Neiswanger<sup>1</sup>, S. O'Brien<sup>2</sup>, B. Nepomunceno<sup>3</sup>, J.C. Murray<sup>2</sup>, M.L. Marazita<sup>1</sup>.* 1) Center for Craniofacial and Dental Genetics, Sch of Dental Med, Univ of Pittsburgh; 2) Dept of Pediatrics, Univ of Iowa; 3) HOPE foundation, Bacolod, Philippines.

Orofacial clefts are common birth defects (1/500- 1/1000) with a complex etiology. Evidence of a relationship between orofacial clefting and dermatoglyphic patterns has been reported in several populations, but has never been investigated in a Filipino population. Dermatoglyphic patterns serve as a marker of the developmental disturbances (environmental and genetic) and increased fluctuating asymmetry (FA) often reported in orofacial cleft study populations. Therefore, we investigated dermatoglyphic patterns in 100 Filipinos with NS CL/P and 89 of their relatives, ascertained from 1998-1999. Patterns were categorized as arch, ulnar loop, radial loop, whorl, or other, by two independent raters, who resolved any discrepancies by re-evaluation. No significant differences were found in the frequency of types of patterns between males and females; this is notable because typically males have more whorl patterns and fewer arches than females. Overall, the frequency of arches was significantly increased and whorls decreased in the affected group ( $p= 0.006$  and  $p= 0.02$ , respectively.) Affected females had significantly more ulnar loops and arches ( $p= 0.037$  and  $p= 0.033$ , respectively), and fewer whorls ( $p < 0.0001$ ) compared with unaffected females. There were no significant differences between affected and unaffected males. As a measure of FA, dissimilarity between pattern types on homologous fingers was quantified, and t-tests were performed. However, no significant differences in dissimilarity scores were found between males and females or between affected and unaffected groups. These results indicate that in this Filipino population, differences in frequency of dermatoglyphic pattern types exist between individuals with orofacial clefts and their unaffected relatives, with the major effect seen in females. This research was supported by NIH grants DE-08559 and DE-13076.

**Sisters with Prenatal Exposure to Alcohol Exhibiting Noonan Phenotype.** *R. Blackston*<sup>1</sup>, *C. Coles*<sup>2</sup>. 1) Div Med Gen, Dept Human Gen, Emory Univ Sch Medicine, Atlanta, GA; 2) Dept Psychology, Marcus Center, Atlanta, GA.

Previous reports have cited isolated cases of Noonan Syndrome phenotype in children with prenatal exposure to alcohol. We report sisters born to a mother who reportedly drank 12 beers daily during the pregnancies and review previous cases in the literature. The proband is a pre-adolescent with short stature, webbed neck, thick hair with low posterior hairline, ptosis, hypoplastic philtrum, thin upper lip, small chin, pectus deformity, mild to borderline developmental delays by educational reports. The adolescent sister has a similar phenotype but reportedly has a normal IQ with visual perceptual motor problems requiring learning disability resources. Chromosome studies of the proband are normal. We have been unable to accomplish desired DNA studies of the PTPN11 gene at 12q (12q21.2q22).

Previous publications have related differentiation between Fetal Alcohol Syndrome (FAS) and other syndromes such as Noonan by facial photographs and facial measurements to be effective. We continue to advocate the full body, Peds Score, we utilize as being more comprehensive lest we miss important findings such as webbed neck, low hair line, pectus deformity. We further stress the necessity of the new DNA studies in Noonan/FAS overlap for objectivity in exact diagnosis in order to provide accurate genetic counseling, and to learn more about this missense mutation.

**Genotype/Phenotype Correlations in Split Hand Foot Malformation (SHFM).** *A.M. Elliott<sup>1</sup>, M.H. Reed<sup>1,2,3</sup>, J.A. Evans<sup>1,3</sup>.* 1) Dept Biochem & Medical Gen, Univ Manitoba, Winnipeg, MB, Canada; 2) Dept Radiology, Children's Hospital, Winnipeg, MB, Canada; 3) Dept Pediatrics and Child Health, Children's Hospital, Winnipeg, MB, Canada.

*Background:* Split Hand Foot Malformation (SHFM) is genetically heterogeneous with 5 loci mapped to date. SHFM is highly variable in its presentation and can occur as an isolated finding or in association with other anomalies.

*Objective:* To perform a clinical epidemiological study of SHFM patients mapped to chromosomes 7, X and 2.

*Methods:* We reviewed the literature of mapped SHFM1 (chromosome 7), SHFM2 (X chromosome) and SHFM5 (chromosome 2) patients. Cases were included if they had: central ray deficiency, a large gap between first and second digits or longitudinal clefting. Cases analyzed included 43 SHFM1, 22 SHFM5 and 36 from the single reported SHFM2 kindred.

*Results:* All SHFM5 patients had chromosomal deletions compared to 30% of SHFM1. None of the SHFM2 cases had deletions, signs of ectodermal dysplasia, craniofacial dysmorphism or other malformations. Almost all SHFM2 cases were males compared to 67% of SHFM1 and 41% of SHFM5. Involvement of both lower extremities was more common with both autosomal loci (SHFM1: both hands affected 35%, both feet 77%; SHFM5: 41% both hands, 95% both feet). Craniofacial findings included: dysplastic ears (35% SHFM1, 41% SHFM5); oral clefts (33% SHFM1, 50% SHFM5); micro/retrognathia (23% SHFM1, 55% SHFM5). Ptosis, microstomia and beaked nose were also common in SHFM5. Deafness, present in 28% of SHFM1, was not seen in SHFM5. Syndactyly, camptodactyly; cardiac defects; seizures and failure to thrive were more common in SHFM5.

*Conclusions:* SHFM1 and SHFM5 patients are at risk for certain craniofacial anomalies and other defects. A proposed animal model for SHFM1 (*Dlx5* and *Dlx6* homozygous mutant mice) shows hindlimb clefting and disturbances of craniofacial development (ears, eyes and branchial arch derivatives) manifesting striking similarities to SHFM1 patients.

**A Kuwaiti boy with multiple joint contracture/Facial dysmorphism "Freeman-Sheldon syndrome":brief clinical report.** *L. Bastaki<sup>1</sup>, R.L. AL-Naggar<sup>1</sup>, S.A. AL-Awadi<sup>1</sup>, R. Usha<sup>2</sup>, M.J. Marafie<sup>1</sup>.* 1) Maternity Hosp, Kuwait Medical Genetic Ctr, Khaldiya, Kuwait; 2) Pediatric Department AL-Jahra Hospital.

We describe a male patient aged one year and seven months-old. He was a product of FT/CS delivery after uneventful pregnancy. The parents were consanguineous/healthy and their previous child was normal. There were two similar conditions, male and female cousins in the family. The boy was failing to gain weight and he had dysmorphic features including long face, full forehead, sunken/small eyes, blepharophimosis, small nose with hypoplastic alae nasi, long philtrum, small mouth, puckered upper lip, ulnar deviation and contracture involving small and large joints. Skeletal survey showed abnormal skull anterior cranial fossa and scoliosis of the spine. Chromosomal analysis showed normal male karyotype (46,xy). The consanguinity of parents and the similar conditions (male/female) raise the possible autosomal recessive mode of inheritance.

**A phenotype reminiscent of FG syndrome in a boy with a submicroscopic deletion of 22q11.2.** *D.J. Aughton, C.E. Harper.* Wm Beaumont Hosp, Royal Oak, MI.

FG syndrome is an X-linked recessive condition characterized in part by cognitive impairment, relative macrocephaly, hypotonia, and severe constipation. Broad forehead and persistent fetal fingerpads are often seen.

Submicroscopic deletion of 22q11.2 (ish del[22][q11.2q11.2]) is a cause of an astonishingly broad array of phenotypes, including DiGeorge sequence, velocardiofacial syndrome, and Opitz G/BBB syndrome. Microcephaly, growth retardation, and cleft palate/bifid uvula are common but not universal findings. Although features suggestive of FG syndrome have been described in association with a submicroscopic *terminal* deletion of 22q (*Clin Genet* 2000;58:483-487), we know of no reports of the joint occurrence of a similar phenotype with ish del(22)(q11.2q11.2). We report such a case.

A 4-year-old boy presented to our clinic in 2002 for further evaluation of developmental delay. He was adopted but was known to have been normally grown at birth. His mother had developmental delay in childhood. The boy's postnatal history was remarkable for hypotonia, severe constipation, and developmental delay. Routine chromosome analysis in 1998 was normal (46,XY). The boy's examination was remarkable for height = ~p25, weight = p25-p50, and OFC = p50-p75 (~p75 for height-age); high, broad, and prominent forehead, without frontal upsweep; prominent supraorbital ridge; downslanting palpebral fissures; intact palate, without clefting of the uvula; overfolded helices; and mild persistent fetal fingerpads.

We considered the boy's findings suggestive of FG syndrome. However, since DNA diagnostics relevant to FG syndrome were not yet clinically available, since the differential diagnosis of FG syndrome includes Opitz G/BBB syndrome (McK \*300000), and since some cases of Opitz G/BBB syndrome owe to ish del(22)(q11.2q11.2), we included FISH studies of 22q11.2 in the boy's further genetic evaluation. The results of these were ish del(22)(q11.2q11.2)(TUPLE1-).

Hence, we propose that a phenotype reminiscent of the FG syndrome is among the many that may be caused by ish del(22)(q11.2q11.2).

**A patient with a genomic deletion encompassing *FOXL2* and *ATR*.** J.J.P. Gille, M.H. de Ru, A.W.M. Nieuwint, J.M. van Hagen. Clinical Genetics, VU University Medical Center, Amsterdam, The Netherlands.

Blepharophimosis-Ptoxis-Epicanthus inversus Syndrome (BPES) is caused by mutations in *FOXL2*. We investigated a boy with BPES who had some additional clinical features: microcephaly, mild mental retardation and growth delay. Chromosome analysis demonstrated a 46,XY karyotype with an interstitial deletion of 3q23-q25. Molecular analysis using VNTR markers showed that the deletion was of maternal origin and encompasses the region between markers D3S1535 and D3S1593. This region contains not only the *FOXL2* gene but also the gene encoding ataxia-telangiectasia and Rad3-related protein (*ATR*). *ATR* has been identified as a candidate gene for Seckel syndrome, an autosomal recessive syndrome characterized by growth retardation, microcephaly and mental retardation. Recently, it was reported that in two Pakistani families a homozygous founder mutation 2101AG segregated with Seckel syndrome (O'Driscoll *et al.*, Nature Genet. 33:497-501, 2003). This mutation results in aberrant splicing of exon 9 although normally spliced mRNA was also detected. It was suggested that only rather mild mutations may cause Seckel syndrome whereas severe mutations are lethal. We hypothesize that also hemizygoty of *ATR* may cause some clinical features of Seckel syndrome and that our patient has a contiguous gene syndrome. The BPES phenotype is caused by deletion of *FOXL2* whereas the other clinical features (microcephaly, mild mental retardation and growth delay) are the result of deletion of *ATR*. Further research is necessary to excluded that other genes in this region contribute to the phenotype of our patient.

**Minor Physical Anomalies in Autism.** *T. Foran<sup>1</sup>, M. Bocian<sup>1</sup>, P. Flodman<sup>1</sup>, J.H. Miles<sup>2</sup>, M.A. Spence<sup>1</sup>.* 1) Division of Human Genetics, Department of Pediatrics, University of California, Irvine, Irvine, CA; 2) The Children's Hospital, University of Missouri-Columbia, Columbia, Missouri.

Minor physical anomalies (MPA) arise from errors in morphogenesis during early embryonic development. Several studies have found an increased frequency of MPA among autistic individuals in comparison to the general population. Increased frequencies of specific MPA in association with autism have also been reported, but no recurring constellation of anomalies has been identified. Using a clinical morphology exam, Miles & Hillman (2000) found that 20% of their autistic sample was dysmorphic (6 MPA). The sex ratios differed significantly between normal and abnormal morphology groups, suggesting different underlying genetic causes between groups.

In this study, clinical morphology exams were used to determine the average number of MPA and frequency of individual MPA in a sample population ( $n = 117$ ) consisting of autistic disorder (79), autistic spectrum (21), and control groups (17). The average number of MPA in the autistic disorder sample is 3.65. The following anomalies occur with increased frequency among those with autistic disorder but not significantly so ( $p < 0.05$ ): increased head circumference, large ears, ptosis, and anomalies of the cranium, forehead, teeth, nose, nipples, columella, philtrum, and mandible. Based on the number of MPA, the sample is classified as morphologically normal (66%), abnormal (20%), or equivocal (14%). The sex ratios in these groups are 5.5:1, 3:1 and 10:1, respectively. When chromosomally abnormal subjects are excluded, the sex ratios are 5.25:1, 5.5:1, and 10:1, respectively. The overall sex ratio in the autistic disorder group is 5.1:1. Assuming that differences in sex ratios result from genetic differences, these findings suggest that autism in abnormal and normal morphology subgroups is causally different. The dysmorphic subgroup may be representative of an autistic population with unidentified chromosomal abnormalities.

**An apparent cluster of gastroschisis: A single center experience.** *S. Chabra, B.D. Hall.* Pediatrics, University of Kentucky, Lexington, KY.

A cluster of 10 neonates diagnosed with gastroschisis at the University of KY in 1996 prompted us to do an analysis to determine possible environmental or genetic causes. A chart review of all neonates admitted to the Neonatal Intensive Care Unit (NICU) with gastroschisis from January 1992- December 1997 was performed. A compilation of maternal and patient demographics were reviewed. A chi-square analysis to determine uniformity of distribution of cases over the 5-year period and an Ederer-Meyers-Mantel test to detect clustering of cases in any one year were performed. Results: 36 neonates with gastroschisis were admitted to the NICU during this period. The mean maternal age was 21.5 years (14-35 years) with the following findings: teenagers (42%), cesarean section (44%), primiparous (66%), smokers (42%), drug users (11%), history of STD (11%), increased amniotic fluid AFP (33%), oligohydramnios (11%) and IUGR (14%). Mean birth weight was 2438g (990-3700g) with mean gestational age of 36 weeks (29-40 wks). Forty-eight percent were males, 54% preterm, 25% had other GI anomalies, and 5% died. The chromosome studies (25%) were normal and there were no familial instances. There was no recurring environmental or drug exposure in the study group. The mothers were from 24 different counties. The 36 cases were not uniformly distributed over the 5 year period (chi square statistic=46.8, degrees of freedom=4,  $p<0.0001$ ). There is no evidence that the cases clustered in any 1 year ( $p=0.99$  for Ederer-Myers-Mantel test). This is one of the few studies of a cluster of babies born with gastroschisis. Many of the mothers were teenagers, primiparous with preterm babies and had an increased frequency of smoking. There is no evidence of temporal clustering and the sparse distribution over 24 different counties showed no spatial clustering. In the majority of cluster studies, there is often initial anxiety as to the etiology of the apparent cluster, however, after epidemiological investigations, frequently such clusters turn out to a random effect. We conclude that the cluster of gastroschisis cases in our study occurred by chance. Epidemiological evaluation of neonates with gastroschisis in different geographic areas of the world would be beneficial.

**Pure trisomy 12p in a mexican patient.** *B. De La Fuente, R. De la Rosa, C. Espinosa.* Genetics Dept., Faculty of Medicine, Universidad Autonoma de Nuevo Leon, Monterrey, NL, Mexico.

**INTRODUCTION:** Trisomy 12p is an unusual chromosome anomaly. There have been only 24 cases of trisomy 12p in the medical literature, 14 with a complete trisomy and 11 were partial, most of them caused by an abnormal segregation of a parental balanced translocation or due to de novo duplications. The clinical characteristics include: retarded psychomotor development, hypoplasia of the middle 1/3 of the face, epicantal folds, flat nose, wide nasal bridge, up-slanting palpebral fissures, long philtrum, full cheeks, thick lower lip, macroglossia, small ears, wide, short hands, 5th finger clinodactyly. **DESCRIPTION:** The patient is a 14 months of age female, product of the first pregnancy among young, non-related parents, and no family medical history of relevance. She had dolicocephaly, peculiar face, up-slanting palpebral fissures, strabismus, high nasal bridge, anteverted nostrils, retrognathia, short neck, hypoplastic right ear, left polythelia, 1st and 2nd toes. A cytogenetic analysis was carried out with GTG banding showing: 46,XX,22,+der(22),t(12;22)(p11;p11)pat. The mother karyotype analysis was 46,XX normal and the father showed: 46,XY,t(12;22)(p11;p11). As a newborn the patient phenotype resembled Down syndrome, between 4 and 6 months of age it resembled that of the Pallister Killian syndrome, both syndromes were discarded by the karyotype analysis. The patient has been in psychomotor stimulation that has improved the muscular tension although the psychomotor delay is present. **CONCLUSIONS:** The medical genetics counseling helps to integrate a diagnosis in order to evaluate and give a better management of associated complications, besides offering prenatal diagnosis to the parents.

**Autism and Dysmorphology: A Valid Observational Measure.** *J. Miles<sup>1</sup>, M. Bocian<sup>3</sup>, J. Farmer<sup>2</sup>, N. Takahashi<sup>1</sup>, M. Spence<sup>3</sup>, S. Braddock<sup>1</sup>, R. Martin<sup>4</sup>, J. Hong<sup>1</sup>.* 1) Dept Child Hlth, Med Gen Div, Univ MO Hosp, Columbia, MO; 2) Dept Health Psych, Univ of MO, Columbia, MO; 3) Div Hum Gen & Birth Defects, Univ of CA, Orange, CA; 4) Wash U School of Med, St. Louis, MO.

Physical dysmorphology is a clear indicator of altered morphogenesis. We previously reported that ~ 15% of individuals with autism had pervasive dysmorphology. To see whether dysmorphology assessments by different dysmorphologists are consistent, four fellowship trained medical geneticists rated the same 30 autistic children with only 73% reliability. We have now formulated a reliable instrument to 1) enumerate the dysmorphic features that denote altered embryogenesis and 2) to score the features so that individuals can be classified as either dysmorphic or non-dysmorphic. The 221 consecutive individuals who met DSM-IV criteria for autism between 1994 & 2001 were studied: 77% (171/221) were nondysmorphic, 12% (26/221) were dysmorphic, 11% (24/221) were equivocal. Individuals with syndromes were excluded. The dysmorphology instrument is organized into a portfolio of 36 physical structure categories. In each category are a list of physical features that reflect altered morphogenesis, can be measured by non-invasive exam, are easy to define or measure and are relatively stable. The features are taken directly from the London Dysmorphology Database feature list (Winter and Baraitser, 1996). Scoring is by category, rather than separate features to measure the breadth of dysmorphology over multiple organ systems. Analysis of the 221 subjects showed that 10 categories distinguished the dysmorphic from nondysmorphic subjects with P values < 3x 10<sup>-6</sup>; 15 additional categories were significant to the 0.05 level. Recoding the 30 exams by this method (without training) increased reliability to 83%. Further validity and reliability studies are in progress. Three features (macrocephaly, ligamentous laxity and deep set eyes) are equally prevalent in all groups and appear to be autism specific. We conclude that a standardized dysmorphology instrument can be user friendly and can classify autistic individuals into distinct subgroups based on their morphogenesis and provide more homogenous groups for genetic study.

**Smith Magenis syndrome - a study of 7 cases in Northern Ireland.** *A. Magee, F. Stewart.* Regional Genetics Service, Belfast City Hosp Trust, Belfast, United Kingdom.

Smith Magenis syndrome (SMS), a contiguous gene syndrome due to interstitial deletions of chromosome 17p11.2, was first described in 1982. The classical features include behavioural problems, major sleep disturbance, developmental delay and self-injury, with a high pain threshold due to varying degrees of peripheral neuropathy. There are characteristic dysmorphic features, although the facial phenotype can show marked variation. Seven sporadic cases of SMS have been identified in the Northern Ireland population (1.8 million) over the last 8 years. There are 2 females and 5 males, ages range from 3 to 36 years. The youngest age of diagnosis was 1 year, and the oldest 34 years. Four of the diagnoses were suspected clinically. All were confirmed by FISH analysis, and 6 had deletions visible on G-banding. All exhibit midface hypoplasia, brachycephaly and a degree of brachydactyly. Five have the characteristic fair hair and blue eyes. All had neonatal feeding problems and hypotonia, and all have speech delay varying from mild in one individual to absent communication skills in one adult. All exhibit behaviour problems including hand and wrist biting, disturbed sleep pattern, and are reported to have high pain thresholds. One boy, now 12 years old, has managed to stay in mainstream education. Four individuals have scoliosis, including one girl with very severe kyphoscoliosis. Three are known to have mild hearing loss. None appear to have renal or cardiac anomalies. Unusual observations include one child with postaxial polydactyly left hand, and 2 have dark hair and sallow skin. The diagnosis of SMS can be difficult on clinical grounds and requires the clinician to be alert to the possibility of SMS, often on the behavioural phenotype alone. These observations, particularly in the adults, give further information on the evolving SMS phenotype.

**Prader-Willi syndrome caused by complex chromosomal rearrangements and uniparental disomy.** *M. Li<sup>1,2</sup>, K.C. Kim<sup>1</sup>, K. Jackson<sup>1,2</sup>, C. Dvorak<sup>1</sup>, H. Andersson<sup>1,2</sup>.* 1) Hayward Genetics Ctr; 2) Department of Pediatrics, Tulane Univ School Med, New Orleans, LA.

We report a novel etiology for Prader-Willi Syndrome (PWS), a de novo Robertsonian translocation of chromosomes 13 and 15 resulting. The 4-year-old boy was referred for genetic evaluation. His medical history was notable for neonatal hypotonia, cryptorchidism and failure to thrive followed by hyperphagia and accelerated weight gain. Physical examination unveiled characteristic facial features of PWS, including bitemporal narrowing, almond-shaped eyes and metopic ridging. His weight was above the 95<sup>th</sup> percentile, and height between the 75<sup>th</sup> and 90<sup>th</sup>. His mental development was delayed. Cytogenetic studies revealed that the patient carried two apparently balanced translocations, t(1;2)(q21;p23) and der(13;15)(q10;q10). FISH with probe SNRPN displayed two copies of the gene. Methylation study uncovered the absence of paternal allele of the SNRPN gene. Maternal chromosomal study performed elsewhere was reported normal. The inheritance pattern of the t(1;2) was not clear since the father was not available for study. These results confirmed the clinical diagnosis of PWS, which had resulted from maternal uniparental disomy (UPD). Maternal UPD accounts for about 25% of PWS patients. Recent publications show that in the majority of PWS patients with UPD, the additional chromosome 15 is due to meiotic nondisjunction. Much less frequently, de novo or inherited isochromosome 15 is the cause. Several cases of familial Robertsonian translocation involving chromosome 15 are also known. The case presented here is the first case of PWS reported to be associated with a de novo Robertsonian translocation involving chromosomes 13 and 15. The mechanisms of the formation of UPD in this patient could be: 1) maternal meiotic nondisjunction (MI or MII) followed by trisomy rescue; 2) a disomic egg fertilized by a nullisomic sperm (in this case, both maternal and paternal nondisjunction are required); or 3) mitotic duplication and paternal nondisjunction. Molecular studies with chromosome 15 microsatellite markers are needed to further define the UPD and better understand the mechanism of its formation.

**Tetra-amelia: Clinical findings in a consanguineous family with four affected siblings.** *S. Niemann<sup>1</sup>, F. Pascu<sup>2</sup>, U. Stahl<sup>2</sup>, U. Aulepp<sup>1</sup>, U. Mueller<sup>1</sup>.* 1) Department of Human Genetics, Justus-Liebig-University, Giessen, Germany; 2) Department of Pathology, Justus-Liebig-University, Giessen, Germany.

Tetra-amelia, the complete absence of all four limbs, is a rare human genetic disorder. In the four families described to date tetra-amelia was most likely inherited as an autosomal recessive trait and the absence of limbs was associated with craniofacial, nervous system, pulmonary, skeletal, and urogenital anomalies. Here we report on a consanguineous Turkish family of Aramaic descent with four affected fetuses displaying autosomal recessive tetra-amelia and urogenital defects associated with craniofacial and skeletal anomalies. In eight pregnancies, tetra-amelia was diagnosed four times prenatally and pregnancies were terminated between the 13th and 20th week of gestation. Autopsy was possible in three of the four affected fetuses: In the first affected fetus, a female, findings were cleft lip with cleft palate, gastroschisis, diaphragmatic defect with malposition of the right lung which was bilobular, agenesis of the left kidney, left supraadrenal gland and spleen, a malformed uterus with only a right, rudimentary ovary and salpinx, and only one umbilical artery. In the second affected fetus, a female, findings were a protrusion and cataract of the left eye, microphthalmia of the right eye, cleft lip with cleft palate, malformed nose with a single naris and choanal atresia, hypoplasia of the pelvis, and atresia of the urethra, vagina and anus. In the third affected fetus, a male, findings were agenesis of left kidney and left supraadrenal gland, hypoplasia of the pelvis, persistence of cloaca, and no external genitalia. The clinical findings of limb agenesis and urogenital defects in all affecteds indicate a link between the early development of the limbs and the urogenital system in humans.

**Rieger syndrome and a de novo (4;17)(q25;q23.3) translocation.** *J. Saraiva<sup>1</sup>, E. Engenheiro<sup>2</sup>, N. Tommerup<sup>2</sup>, E. Matoso<sup>3</sup>, I. Marques<sup>3</sup>.* 1) Servico Genetica Medica, Hospital Pediatrico, Coimbra, Portugal; 2) Department of Medical Genetics, Wilhelm Johannsen Center for Functional Genome Research, University of Copenhagen, Denmark; 3) Laboratorio de Citogenetica, Instituto de Biologia Medica, Faculdade de Medicina de Coimbra, Portugal.

Rieger syndrome has an autosomal dominant inheritance due to mutations in the PITX2 gene in 4q25q26. There is a second locus ascribed to 13q14. Several case reports describe Rieger syndrome in patients with chromosomal abnormalities. We report a further case of Rieger syndrome with chromosomal breakpoint in 4q25 in a de novo reciprocal translocation t(4;17)(q25;q23.3). A deletion of approximately 2 Mb has been found at the 4q25 breakpoint. We discuss the possible clinical classification of Rieger syndrome in types 1 and 2 related to the 4q25 and the 13q14 loci, the reasons why reporting of chromosomal abnormalities with breakpoints in 4q25 is common and the molecular differences in PITX2 mutations that are the cause of Rieger syndrome or isolated iridogoniodysgenesis.

**Cryptic interstitial deletion of chromosome 16q12 : a novel recognizable deafness/mental retardation syndrome ?**

*M. Rio*<sup>1</sup>, *S. Lyonnet*<sup>2</sup>, *M. Prieur*<sup>2</sup>, *S. Briault*<sup>3</sup>, *M. Vekemans*<sup>2</sup>, *A. Munnich*<sup>1</sup>, *L. Colleaux*<sup>1</sup>. 1) INSERM U393, Hosp Necker-Enfants Malades, Paris, France; 2) Departement de genetique, Hosp Necker-Enfants Malades, Paris, France; 3) INSERM U316, Hosp Bretonneau, Tours, France.

Syndromic hearing loss represents an extremely heterogeneous group of conditions (more than 300 entries) which includes mendelian diseases such as Fountain syndrome or Juberg-Marsidi syndrome, and chromosomal anomalies including deletions of chromosomes 1p36 or Xq21. Here we report a novel form of syndromic hearing loss ascribed to an interstitial deletion on chromosome 16q11.2-q12.1 in three sibs with severe mental retardation, deafness, post-natal growth retardation, hand and foot anomalies, dysplastic ears and distinctive facial features. FISH and molecular analyses showed that the three children carried the same interstitial deletion of chromosome 16 encompassing SALL1, the gene which accounts for a syndromic form of hearing loss, Townes-Brocks syndrome (TBS). TBS is an autosomal dominant inherited syndromic hearing loss associated with anus malformation, hand and foot anomalies. Interestingly, the clinical presentation of our patients is relatively different from TBS phenotype. We propose therefore that the del16q12 is a novel clinical recognizable deafness/mental retardation syndrome.

**Identification of eleven possible regions for NSCLP loci.** *S.H. Blanton*<sup>1</sup>, *T. Bertin*<sup>2</sup>, *S. Patel*<sup>2</sup>, *S. Stal*<sup>3</sup>, *J. Mulliken*<sup>4</sup>, *M.E. Serna*<sup>2</sup>, *J.T. Hecht*<sup>2</sup>. 1) University of Virginia Hlth Sci Ctr, Charlottesville, VA; 2) University of Texas Medical School, Houston, TX; 3) Texas Children's Hospital, Houston, TX; 4) Children's Hospital, Boston, MA.

Nonsyndromic cleft lip with or without cleft palate (NSCLP), a common birth defect affecting 1/700 live births and 4,000 newborns/year in the United States, is associated with short and long-term morbidity. As such, it has significant impact on the health care system. NSCLP is a complex disorder that results from the interaction of genetic and environmental factors that are slowly being defined. Recently, two genomic scans have suggested a number of regions that may contain NSCLP loci. However, there remain a number of additional viable candidate genes and regions that have not been sufficiently investigated. These include chromosomal translocations in patients with NSCLP, growth factor genes, metalloproteinase (MMP) and transcription factor (patterning) genes, including those in the WNT family. In this study, we have evaluated regions identified by a previously published genomic scan of affected sib pairs in addition to screening the 10p13 chromosomal translocation region associated with NSCLP, MMP genes clustered on chromosomes 1p36, 11q22.3, 16p13.3 and 16q12-13, and the region containing the WNT5A gene on chromosome 3p21. Using linkage disequilibrium methods as implemented in Genehunter2 and PDT in our sixty-five multiplex families, we have found evidence of association for eight chromosomal regions from the genome scan. Four of these regions, 2q37, 11p12-14, 12q13 and 16p13, have also been identified in second genomic scan of multiplex families from China. In addition, markers from three of the candidate regions, 10p13, 16p13.3 (MMP25) and 3p21.2, yielded findings that are sufficiently significant to warrant closer investigation. These results suggest chromosomal regions that should be targeted in order to identify NSCLP loci. We are currently examining additional markers in these regions and evaluating candidate genes.

**Phenotypic and Molecular characterization of distal 19q trisomy detected by FISH.** *J. Ganesh<sup>1</sup>, M.K.*

*Maisenbacher<sup>1</sup>, L. Medne<sup>1</sup>, E.C. Moore<sup>1</sup>, M. Nimmakayalu<sup>1</sup>, T. Moshang<sup>2</sup>, E.H. Zackai<sup>1</sup>, N.B. Spinner<sup>1</sup>.* 1) Division of Human Genetics and Molecular Biology The Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Division of Endocrinology, The Children's Hospital of Philadelphia.

The use of sub-telomeric FISH probes to augment cytogenetic studies has resulted in the identification of previously unrecognized chromosomal anomalies not detected by conventional banding techniques. Sub-telomeric FISH studies led us to identify 5 patients in three families with trisomy of the subtelomeric region of 19q. In two families, the 19q was translocated to the short arm of an acrocentric chromosome. Family 1 was segregating a cryptic t(13;19)(p12;q13.3) in 3 generations. Six individuals were balanced carriers and 3 individuals (2 siblings and their maternal uncle) inherited the der(13), which resulted in trisomy for distal 19q. Affected individuals were non-dysmorphic, had mild to moderate developmental delay, attention deficit disorder (ADD) and short stature. Growth hormone (GH) deficiency was identified in the two siblings. The proband in Family 2 had an unbalanced translocation with der15 t(15;19)(p13;q13.3) (mat), with mild developmental delay, hypotonia, ADD, growth failure and short stature with normal GH levels. She also had joint hypermobility, ptosis, epicanthic folds and down slanted palpebral fissures, which were also seen in her mother. The proband in Family 3 had a der14t(14;19)(Tel 14q-, Tel 19q+). His height and weight were normal but he had moderately severe cognitive and behavior problems. The phenotype in this cohort was considerably milder than that which has been associated with cytogenetically visible trisomy 19q. FISH mapping with chromosome 19 specific BAC clones was done to map the breakpoints of these translocations. The patient without short stature had the most distal breakpoint, between 19q13.43 to qter, while individuals with short stature had breakpoints between 19q13.33 and 19q13.41 implying this to be a critical region for growth. Further molecular studies are needed to characterize this region, specifically regarding GH deficiency.

**Mutation Analysis of the VSX1 Gene in Patients with Posterior Polymorphous Corneal Dystrophy.** *A.J. Aldave<sup>1</sup>, V. Yellore<sup>1</sup>, N. Udar<sup>2</sup>, K. Small<sup>2</sup>.* 1) Cornea Service, The Jules Stein Eye Institute, Los Angeles, CA; 2) Retina Service, The Jules Stein Eye Institute, Los Angeles, CA.

**Purpose:** To screen for mutations in the VSX1 gene, in which mutations have been previously identified in patients with posterior polymorphous corneal dystrophy (PPMD). **Methods:** DNA extraction, PCR amplification and direct sequencing of the VSX1 gene was performed in 10 affected patients (9 families) as well as in unaffected family members and healthy control subjects. **Results:** No coding region mutations were identified in affected patients. However, a synonymous substitution (Ala182Ala) was identified in 4 patients (4 families), present in the homozygous state in 1 patient. Four affected patients (4 families) and 1 unaffected patient demonstrated a duplication in the 5'UTR (11\_20dup). **Conclusion:** No VSX1 gene coding region mutations were identified in PPMD patients, suggesting other genetic factors are involved in this autosomal dominant disorder.

**Molecular characterization of chromosomal breakpoint in a patient with CHARGE Syndrome.** *S.R. Lalani<sup>1</sup>, A. Safiullah<sup>1</sup>, S.D. Fernbach<sup>1</sup>, D.M. Martin<sup>3</sup>, J.W. Belmont<sup>1,2</sup>.* 1) Dept of Genetics, Baylor College of Medicine, Houston, TX; 2) Dept of Pediatrics, Baylor College of Medicine, Houston, TX; 3) Dept of Pediatrics and Human Genetics, University of Michigan, MI.

CHARGE syndrome is characterized by congenital heart disease, coloboma of the eyes, choanal atresia, retardation of mental and somatic development, genital abnormalities, ear abnormalities and/or deafness. While significant progress has been made in the clinical delineation of this syndrome, the molecular basis for the disorder remains unknown. Rare familial cases, increased paternal age, concordance between monozygotic twins, and reports of patients with unbalanced chromosomal anomalies suggest a genetic basis for CHARGE syndrome. We have been studying a child with CHARGE syndrome who has an apparently balanced de novo t(2;7)(p14;q21.11) chromosomal translocation. The child has bilateral choanal atresia, absence of semicircular canals, cranial nerve dysfunction, growth retardation, and genital hypoplasia, features consistent with CHARGE syndrome. We hypothesized that this apparently balanced translocation interrupts a gene with extreme pleiotropy, or contributes to the phenotype secondary to a positional effect. We have used fluorescence in situ hybridization (FISH) to identify the breakpoints in this patient and used BAC and PAC clones from the genome assembly database. By FISH analysis, we have identified the clones that span the breakpoints on both 2p14 and 7q21.11 loci. Using a long range PCR product of 9 kb as a FISH probe, we have further characterized the breakpoint within this region and identified a gene disrupted in this patient. Efforts are currently underway to sequence this gene in sixty patients with CHARGE syndrome. This study has facilitated characterization of a gene that may be involved in a subset of patients with CHARGE syndrome.

**Deletion 2q22-q23 syndrome: a contiguous gene syndrome including clinical features manifested in SIP1 deficiency.** *N. Ishihara*<sup>1,2</sup>, *K. Yamada*<sup>2</sup>, *Y. Yamada*<sup>2</sup>, *K. Miura*<sup>3</sup>, *J. Kato*<sup>4</sup>, *N. Kuwabara*<sup>5</sup>, *Y. Kobayashi*<sup>6</sup>, *T. Kumagai*<sup>3</sup>, *M. Nagaya*<sup>4</sup>, *N. Wakamatsu*<sup>2</sup>. 1) Dept Pediatrics, Nagoya Univ Sch Medicine, Nagoya, Aichi, Japan; 2) Dept Genetics, Inst Dev Res, Aichi Human Service Ctr, Aichi, Japan; 3) Dept Pediatr Neurol, Centl Hosp, Aichi Human Service Ctr, Aichi, Japan; 4) Dept Pediatr Surg, Centl Hosp, Aichi Human Service Ctr, Aichi, Japan; 5) Dept Pediatr Cardiol, Gifu Pref Hosp, Gifu, Japan; 6) Dept Pediatrics, Takuto Rehabil Ctr for Children, Miyagi, Japan.

SIP1 deficiency is caused by heterozygous mutations in *ZFHX1B* or deletions at 2q22 containing *ZFHX1B* resulting in profound mental retardation, delayed motor development, microcephaly, facial dysmorphism with a variety of combinations of epilepsy, congenital heart disease, Hirschsprung disease and failure of development of midline structures. However, there are several points to be elucidated. 1) What is the size of the deletions located at 2q22, if the patients present with the same clinical features of SIP1 deficiency. 2) What kind of clinical features might be newly manifested if deletions span 2q23-q24 as a contiguous syndrome. To address these questions, we analyzed clinical features and determined precise breakpoints of eight patients underlying deletions at 2q22-q24. We identified 0.2- to 5.1-Mb deletions including *ZFHX1B* at 2q22 from six patients presenting similar clinical features of SIP1 deficiency. In contrast, 8.8-Mb and 10.5-Mb deletions at 2q22-q23 and 2q22-q24 were identified in two cases presenting with more severe clinical features including cleft palate and complicated heart diseases. It is noteworthy that four of the patients with a common deletion at 2q23 had both clinical features (Lurie et al, Genet Couns. 1994; 5:11-14). Thus, loss of heterozygosity of genes located at 2q22-q23 causes a contiguous gene syndrome including features of SIP1 deficiency, cleft palate and heart disease.

**Assessment of association between TBX1 variants and haplotypes with manifestation of congenital heart defects in 22q11.2 deletion patients.** *A. Rauch<sup>1</sup>, K. Devriendt<sup>4</sup>, A. Koch<sup>2</sup>, R. Rauch<sup>6</sup>, M. Gewillig<sup>5</sup>, C. Kraus<sup>1</sup>, M. Weyand<sup>3</sup>, H. Singer<sup>2</sup>, M. Hofbeck<sup>6</sup>, A. Reis<sup>1</sup>.* 1) Institute of Human Genetics, Friedrich-Alexander Univ, Erlangen, Germany; 2) Departments of Pediatric Cardiology of the Friedrich-Alexander University Erlangen-Nuremberg,; 3) Department of Heart Surgery of the Friedrich-Alexander University Erlangen-Nuremberg,; 4) Centre for Human Genetics, Leuven, Belgium; 5) Centre for Pediatric cardiology, Leuven, Belgium; 6) Department of Pediatric Cardiology of the University of Tuebingen, Germany.

Deletion 22q11.2 is a major cause of congenital heart disease. However, presence of the deletion does not allow to predict the phenotype as patients with a 22q11.2 deletion usually show a broad clinical variability. TBX1 was shown by knockout mice experiments to play a role in congenital heart defects and therefore constitutes a candidate gene for a modifier in deletion 22q11.2 patients, in terms of uncovering of recessive mutations by hemizyosity. Thus we sequenced the entire coding region of TBX1 in a total of 174 patients with 22q11.2 deletion and detected 16 different variants, 7 of which represent common SNPs. Up to 96 dizygous control persons were additionally typed for these 16 variants. A combined approach with pair wise LD calculation on 174 due to hemizyosity naturally given haplotypes and analysis of background haplotypes of rare variants in a total of 366 chromosomes allowed us to define a reliable haplotype structure consisting of 3 LD blocks within the coding region and a further LD block including the 5'UTR exon 1. 14 of the 16 variants as well as the common haplotypes were detected at equal frequencies in deletion patients with and without CHD as well as in healthy controls. Thus we excluded common TBX1 variants or haplotypes as modifiers of expression of congenital heart defects in 22q11.2 deletion patients. Nevertheless, one 9 bp deletion was not detected in normal controls and segregated CHD in one family and might therefore constitute a causative factor for CHD in this family, as might apply for one further variant in one patient with PA-VSD, which was not detected in normal controls and which was the only variant to affect a conserved nucleotide.

**Analysing the molecular basis of Proteus Syndrome by genomic sequencing of candidate genes *PTEN*, *GPC3* and *p57*.** I. Thiffault<sup>1,2,3</sup>, C.E. Schwartz<sup>4</sup>, V. Der Kaloustian<sup>1</sup>, W.D. Foulkes<sup>1,2,3</sup>. 1) Department of Human Genetics, McGill University, Montreal, PQ, Canada; 2) Research Institute of the McGill University Health Centre, McGill University, Montreal, PQ, Canada; 3) Cancer Prevention Centre, Sir M B Davis-Jewish General Hospital, McGill University, Montreal, PQ, Canada; 4) Greenwood Genetics Center, Greenwood, SC, USA.

Proteus syndrome (PS) is a rare and complex disorder comprising an association of asymmetrical gigantism, epidermal naevi, vascular malformations, hamartomas, lipomas and hyperstosis. Since the first description of the syndrome by Cohen and Hayden, many hypotheses have been proposed to explain its occurrence. The most plausible is Happle's somatic mosaic hypothesis, but no somatic mutation in candidate genes have been reported to be clearly involved in PS. Mutation studies provided contradictory results on the association of germline mutations in children with Proteus and Proteus-like disorders: in a total of 48 patients with PS (n=42) or PS-like (n=6) syndrome, 7 have been reported to harbour a germline *PTEN* mutation (4 of 7 are PS-like). The classification of PS as a *PTEN*-hamartomatous syndrome remains controversial and given these data, the molecular basis of PS remains elusive. In this study, affected and unaffected tissues from 8 patients diagnosed with PS were screened by direct sequencing to determine the role of *PTEN*, *GPC3* and *p57* in PS development. Mutation analysis did not reveal any mutation in the coding sequence of *PTEN*, *GPC3* and *p57* in these PS patients. These mutational findings indicate that genomic alterations in *PTEN*, *GPC3* and *p57* are unlikely to play a major role in the pathogenesis of PS. Giving these results, a genome-wide, 10cM 388 marker microsatellite screen is currently underway to uncover putative somatic genomic microdeletions or other rearrangements by comparing the allelotype of the affected and unaffected tissues from PS patients. The aim of this molecular study is to shed light new understanding of this rare disorder.

**Identification of a novel locus for nanophthalmos, a form of severe hyperopia.** *O.H. Sundin<sup>1</sup>, J.M. Yang<sup>1</sup>, G.S. Leppert<sup>1</sup>, S. Dharmaraj<sup>1,2</sup>, I.H. Maumenee<sup>2</sup>, E. Weinberg<sup>1</sup>, E.D. Silva<sup>1</sup>.* 1) Developmental Genetics, Department of Ophthalmology, Johns Hopkins School of Medicine, Baltimore, MD; 2) Center for Hereditary Eye Diseases, Department of Ophthalmology, Johns Hopkins School of Medicine, Baltimore, MD.

Nanophthalmos, also known as uncomplicated microphthalmia, is a rare genetic disorder of the human eye characterized by extreme bilateral hyperopia (farsightedness), with a refractive error greater than +8 diopters. Although the cornea and lens are typically of normal proportions, the optic axis of the eye is unusually short, in the range of 14-20mm, relative to the 23-24mm axis characteristic of a normal adult eye. With this condition, angle-closure glaucoma and detachment of the retina are common complications in adults. We have identified a large kindred with 9 affected individuals distributed in a recessive pattern of inheritance over 4 sibships. The affected show classic features of the disorder, with refractions ranging from +10 to +25 diopters, thickened sclera, and narrow angles between the iris and cornea. The 4 most severely affected have either extensive or incipient retinal detachments. A whole genome screen using tandem repeat polymorphisms was carried out with 5 affected and 9 unaffected siblings. We obtained linkage to a novel 3 cM interval on chromosome 11q with a maximum LOD score of 6.8. This interval is distinct from the previously described dominant NNO1 nanophthalmos locus on chromosome 11. Identification of mutations underlying this disorder promise insight into how embryonic and postnatal development of the eye normally achieve an organ with remarkably standard shape and optical parameters.

**An Unusual Presentation of Angelman Syndrome due to an Imprinting Error on Chromosome 15.** *L. Steele<sup>1</sup>, T. Wright<sup>1</sup>, L. Oman-Ganes<sup>2</sup>, R. Munn<sup>2</sup>, D. Mills-Tettley<sup>2</sup>, I. Teshima<sup>1</sup>, T.L. Stockley<sup>1</sup>, P.N. Ray<sup>1</sup>.* 1) Dept Paed Lab Med, Hosp Sick Children; 2) Toronto, ON, Canada.

Prader Willi (PWS) and Angelman (AS) syndromes are clinically distinct but both are due to aberrant expression of genes at 15q11-13. PWS is caused by lack of paternally imprinted genes at 15q11-13, whereas AS is due to the lack of maternally imprinted genes. Each disorder has a frequency of about 1 in 20,000 live births. Children with PWS often present in the newborn period with hypotonia, obesity and developmental delay. In contrast, AS is difficult to diagnose in the newborn period since the characteristic behavioral features are usually recognized after the first few years of life. Approximately 70% of PWS and AS cases are due to parent-specific deletions in the 15q11-q13 region. Uniparental disomy (UPD) accounts for ~25% of PWS cases (matUPD) and 3-5% of AS cases (patUPD). Specific defects in genetic imprinting are less common, having been described in ~5% of PWS and AS cases. These defects are detected by molecular testing for SNRPN methylation status, followed by STR marker analysis to determine the etiology of the disorder. Here we report a 14 month old female presenting with severe hypotonia and developmental delay without other characteristic features of PWS. The child was delivered to a 42-year-old primigravida following artificial insemination for a 3-year history of infertility. Blood chromosome analysis revealed a 46,XX karyotype. Molecular testing for PWS was requested due to unexplained hypotonia. SNRPN methylation analysis revealed the presence of a paternally imprinted chromosome 15 but no maternally imprinted chromosome 15. This indicates that the patient has AS and not PWS. STR analysis revealed biparental inheritance of chromosome 15, indicating that the maternally derived chromosome 15 that the child inherited from the mother has a paternal imprint producing a rare, atypical clinical presentation of severe hypotonia and developmental delay. This finding suggests young children with hypotonia should be evaluated with molecular SNRPN testing in order to detect this unusual clinical presentation.

Program Nr: 708 from 2003 ASHG Annual Meeting

**Germline mosaicism of a novel mutation of *UBE3A* in Angelman syndrome.** S. Saitoh, K. Hosoki, K. Takano, A. Sudo. Dept Pediatrics, Hokkaido Univ Sch Medicine, Sapporo, Japan.

Approximately 10% of Angelman syndrome (AS) cases are caused by a mutation of *UBE3A*. The *UBE3A* gene shows tissue-specific imprinting and only the maternally derived allele is expressed in certain areas of the brain, including the hippocampus and cerebellum. More than 50 mutations of *UBE3A* have been described, and these mutations have been either maternally inherited or have arisen *de novo*. We have investigated two siblings with a typical AS phenotype. Lymphoblastoid cell lines were established from the affected sibs, a non-affected sib, and the parents. PCR amplification of genomic DNA and direct sequencing of all coding exons and flanking sequences identified a novel splicing mutation of *UBE3A* (IVS14-2AG). RT-PCR using RNA from lymphoblastoid cells revealed the skipping of exon 15 that created a translational frameshift and premature termination. This mutation was not present in the mother or father, although we could only examine lymphoblastoid cells. Further genetic studies revealed that the affected sibs inherited identical haplotypes from the mother as well as from the father, while the non-affected sib inherited different haplotypes from the mother and father. Thus, the parental origin of the chromosome 15 that carried the mutation could not be determined. Nonetheless, the combined data indicate that the mutation most likely is present as maternal germline mosaicism. This is the first report of germline mosaicism for a mutation of *UBE3A*, and has important implications for genetic counseling in Angelman syndrome.

**Yellow Nail Syndrome: Evidence of autosomal recessive trait.** *L.V. Furtado<sup>1</sup>, C.D. Martinhago<sup>1</sup>, J.C. Bruno<sup>3</sup>, J.M. Pina Neto<sup>1,2</sup>*. 1) Medical Genetics, HCFMRP, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil; 2) Dept. Genetics, School of Medicine of Ribeirao Preto- University of Sao Paulo, Brazil; 3) Dept. of Pneumology, HCFMRP- University of Sao Paulo, Brazil.

The yellow nail syndrome (153300 OMIM) is a rare disorder delineated by Sammon and White (1964), characterized by slow growing yellow dystrophic nails and adult onset peripheral lymphoedema. Associated abnormalities include: bronchiectasis, chronic recurrent pleural effusion, chylous ascites, immune deficiency, chyluria, nephrotic syndrome, chronic sinusitis and other respiratory infections. Inheritance is autosomal dominant, although there are sporadic cases reported. However, Kamatani et al (1978) reported two adult brothers, the offspring of first cousins, with apparent yellow nail syndrome and mental retardation. The condition differed from classical yellow nail syndrome in that mental retardation was present and the parents were normal, suggesting recessive inheritance in this particular family. We reported a family in which a 36 year- old man, the second sib of a consanguineous marriage, was found to have neonatal onset slow growing, excessively curved, yellowish nails. At 29 years old was detected oedema of lower limbs, feet and hands; intermittent oedema of face; chronic sinusitis; chronic recurrent pleural effusions; bronchiectasis and immune deficiency, without intellectual handicap. Neither hepatic nor renal failures were found. Review of his family pedigree reveals that he was the most affected sib. Immediate family history is notable for normal parents, but not his two sisters which had neonatal onset typical nails changes; adult onset peripheral oedema and associated pulmonary features. One of these women had two offspring that were spared, suggesting a novel form of yellow nail syndrome with presumed autosomal recessive inheritance and normal intelligence. Supported by FAEPA-HCFMRP, University of Sao Paulo.

**Pseudodominance in pseudoxanthoma elasticum - a common phenomenon: Implications for genetic counseling.**

*F. Ringpfeil, K. McGuigan, H. Kozic, J. Utto.* Dept of Dermatology and Cutaneous Biology, Jefferson Medical College, Philadelphia, PA.

Pseudoxanthoma elasticum (PXE), a heritable disorder affecting skin, eyes and cardiovascular system with protean manifestations, has an estimated incidence of 1 in 70,000. Through mutation analysis in the ABCC6 gene, we and others have previously established the inheritance pattern as autosomal recessive with complete penetrance. However, several clinical investigators have reported dominant inheritance in families with affected individuals in two generations. Also, obligatory heterozygous carriers may have a very limited phenotype, often reflected only by positive histopathology from predilection sites of skin or by asymptomatic eye findings. Within our cohort of over 65 PXE families, we have ascertained several families that meet both criteria. We have haplotyped these families using microsatellite markers and SNPs spanning 3 cM around the ABCC6 locus and we have sequenced the entire coding region of ABCC6 in affected individuals and presumed carriers with a limited phenotype. All affected individuals and those with a limited phenotype harbor two mutated alleles, most mutations being private and novel. In one of these families, consanguinity in previous generations was detected through haplotype analysis. In the other families, affected individuals in two generations carried two sets of affected alleles, with a total of three different mutations. Haplotype analysis confirmed that the affected alleles were inherited independently. These data emphasize that PXE is an autosomal recessive disease that requires mutations in both alleles even for minimal signs of disease. Phenotypic variation between generations may be attributed to the consequences of mutations at the level of the encoded protein, MRP6. Furthermore, our findings imply a much higher prevalence of PXE than previously estimated, thus impacting on genetic counseling and disease prevention. It may therefore not be unreasonable to subject clinically unaffected individuals at risk for PXE to a skin biopsy to screen for subclinical disease.

**Split hand/split foot malformation with sensorineural deafness: a case report.** *F. McAuliffe*<sup>1</sup>, *S. Blaser*<sup>2</sup>, *B. Papsin*<sup>2</sup>, *D. Chitayat*<sup>1</sup>. 1) Obstetrics & Gynecology, Mount Sinai Hospital, Toronto, Ontario, Canada; 2) Hospital for Sick Children, Toronto, Canada.

Ectrodactyly and sensorineural deafness is a rare association. We report an isolated case with this combination. The patient is a 1-year-old boy affected with four limb ectrodactyly and severe sensorineural deafness. He is the only member in his extended family with such a defect. The limb abnormality was detected antenatally. He was born after a full-term pregnancy and the delivery was spontaneous and vaginal with birth weight of 4250 gr. His karyotype is normal and male and neither dysmorphic features nor ectrodermal defects are evident. His general development is age appropriate apart from language. On the right hand 2nd and 3rd fingers are absent and on the left hand the 3rd finger is missing, with syndactyly of 4th and 5th fingers. On the right foot the 2nd and 3rd toes are absent and on the left foot the 2nd toe is missing with syndactyly of 3rd and 4th toes. He is completely deaf in the left ear and some hearing is present in the right ear. MRI reveals fused bones of the inner ear and a short cochlea. He received a cochlear implant in his right ear at a year of age. Four loci for split hand-split foot malformation have been mapped: SHFM1 on chromosome 7q21, SHFM2 on chromosome Xq26, SHFM3 on chromosome 10q24, SHFM4 recently found caused by mutations in the p63 gene, a homologue of the archetypal tumor suppressor TP53, on chromosome 3q27. This boy does not have the ectodermal defects or facial clefting, thus making the diagnosis of ectrodactyly-ectodermal dysplasia-clefting syndrome unlikely. An autosomal dominant ectrodactyly with sensorineural deafness was reported recently (1,2) and this case may represent a new dominant mutation. References 1. Mishra P, Muranjan M, Bharucha BA. Autosomal dominant ectrodactyly with sensorineural deafness. *Clin Dysmorphol* 2000 Apr;9(2):119-21. 2. D Tackels-Horne, A Toburen, E Sangiorgi, F Gurrieri, X de Mollerat, R Fischetto, F Causio, K Clarkson, Re Stevenson and Ce Schwartz. Split hand/split foot malformation with hearing loss: first report of families linked to the SHFM1 locus in 7q21. *Clinical Genetics*; 59 (1): 28.

**Genome Scan of Cleft lip with or without Cleft Palate (CL/P): Part I. Broadening the Phenotype to Include Lip Muscle Defects.** *S.M. Weinberg<sup>1</sup>, K. Neiswanger<sup>1</sup>, M.P. Mooney<sup>1</sup>, M.E. Cooper<sup>1</sup>, T.H. Goldstein<sup>1</sup>, A. Bowen<sup>2</sup>, R.A. Martin<sup>3</sup>, M.L. Marazita<sup>1</sup>.* 1) Center for Craniofacial and Dental Genetics, School of Dental Medicine, University of Pittsburgh; 2) Radiology Division, Childrens Hospital of Pittsburgh; 3) Division of Medical Genetics, St. Louis Childrens Hospital.

To date, genetic studies of CL/P have defined affection status narrowly, resulting in a potential loss of power for gene mapping studies if there are subclinical phenotypes present in non-cleft carriers of CL/P susceptibility genes. Studies using ultrasound have found a higher frequency of subclinical defects in the lip muscles of non-CL/P individuals within CL/P families compared to controls. A genome wide scan using 392 markers, with average spacing of 8.9 cM, was performed on 24 CL/P multiplex families from Western PA (122 family members genotyped by CIDR). Analyses were performed for both narrow and broad affection status definitions, with the broad definition including both overt CL/P plus subclinical lip muscle defects. There were 36 affected family members under the narrow definition, and 44 under the broad. Two-point and multipoint parametric linkage analyses were performed by the method of LOD scores. Results of the genome scan under the narrow affection status definition found suggestive LOD scores (between 1 and 2) on chromosomes 2, 6, 7, 8, 10 and 18. The highest two-point LOD was 1.97 on Chr 2. The highest multipoint LOD was 1.36 on Chr 6. Results of the genome scan with the broad affection status differed substantially. LODs greater than 1 were seen on chromosomes 1, 2, 5, 6, 8, 10, and 17. The highest two-point LOD was again found on Chr 2, but the LOD increased to 2.32. The highest multipoint LOD was 1.83 on Chr 17. Compared to the results of the narrow definition scan, seven LODs increased to over 1, seven decreased below 1 and three initially over one increased further. These results suggest that several of the genes responsible for CL/P also lead to lip muscle defects, and highlight the potential importance of including subclinical markers in gene mapping studies of CL/P. Supported by NIH grants DE13076 & RR00084; genotyping provided by a grant from the Center for Inherited Disease Research.

**POF in a mentally retarded girl with epilepsy and duplication of p arm of chromosome 22.** *M.E. Ahmad, R. Dada, K. Kucheria.* Division of Genetics, Department of Anatomy, All India Institute of Medical Sciences (AIIMS), New Dehli-110029, India.

There is an increased risk of reproductive disorders in women with epilepsy. Generalized tonic clonic seizures are associated with hypothalamic hypogonadism and polycystic ovarian disease. Premature Ovarian Failure (POF) affects 1% of women and is known to be caused by chromosomal abnormalities. Chromosomal rearrangements are also the most frequent cause of mental retardation. We report a case of a 20 year old mentally retarded girl with POF and history of epilepsy. On cytogenetic analysis of 30 G-banded metaphases we found duplication of 22p (46,XX dup22p). The proband had poorly developed secondary sexual characters. The FSH and LH levels were in the normal range and USG showed complete absence of ovarian follicles and the uterus was small in size. The proband was a product of non consanguineous marriage and there was no family history of delayed menarche or epilepsy. Though it is well known that chromosomal abnormalities lead to POF and mental retardation, it is important to understand their correlation and analyse more such cases to understand if genes controlling mental development and ovarian development are linked. Thus understanding the genetic basis of these disorders is important in order to counsel these cases and manage them most appropriately.

**Phenotypic characterisation of ring chromosome 22.** *S. Curran*<sup>1</sup>, *A. Jeffries*<sup>2</sup>, *J. Powell*<sup>2</sup>. 1) SGDP Research Ctr, Inst Psychiatry, London, United Kingdom; 2) Neuroscience, Inst Psychiatry, London, United Kingdom.

Ring chromosome 22 (r22) is a rare human cytogenetic abnormality. Individuals with ring chromosome 22 exhibit a phenotype of global developmental delay with severe delay or absence of speech, hypotonia and more variable minor dysmorphic features. Autistic disorders as well as seizures have also been reported. Formation of the ring chromosome is assumed to arise from breakage and subsequent fusion of both chromosome arms to generate a ring chromosome with concomitant loss of short and long arm sequences. Hemizygoty of genes on the long arm of chromosome 22 is assumed to determine the phenotype. We have determined the breakpoints of 30 phenotypically characterised individuals, the largest reported collection of r22 patients to date. Families were mailed questionnaires, a disposable camera and buccal swab kits for DNA sampling of affected individuals and their natural parents where available. A clinical history questionnaire, gathered information on basic demographic information as well as a detailed history of the pregnancy, birth, developmental, medical and educational history of the child. The majority of the children in this study were globally developmentally delayed, with severe impairment in functioning. In order to try to quantify the developmental level achieved we used the PIP developmental charts which focus on 'milestones' of development in the five main areas: physical development, social development, eye-hand development, development of play, and language development. The 40-item Social Communication Questionnaire (SCQ), was used to screen for autistic symptoms. Microsatellite analysis of ring 22 individuals and their parents using twenty two published and two unpublished markers was used to determine the 22q breakpoints of the r(22) chromosomes. Even though, 22q DNA loss varied from less than 69 kb up to 10.1 Mb or <0.15% to 21% of the total chromosome length we found no significant association of breakpoint positions with dysmorphological features, developmental attainment, medical problems or behavioural features of the r22 individuals. We discuss the reasons for this and note a high prevalence of autistic behaviours in this group.

**Infantile Systemic Hyalinosis in a patient with chromosome 22 inversion.** *N. Macias-Gomez*<sup>1,2</sup>, *L. Arnaud*<sup>1,3</sup>, *J.A. Nastasi*<sup>1,2</sup>, *J. Durán*<sup>1,2</sup>, *H. Gomez-Sanchez*<sup>2</sup>, *R.M. Davalos-Pulido*<sup>2</sup>, *A.I. Vásquez*<sup>2</sup>, *J.E. Garcia-Ortiz*<sup>4</sup>, *M.P. Gallegos-Arreola*<sup>3</sup>, *L.E. Figuera*<sup>2</sup>. 1) División de Genética, CIBO, IMSS, Guadalajara, Jalisco, México; 2) Doctorado en Genética Humana, UDG, Guadalajara, Jalisco; 3) División de Medicina Molecular, CIBO, IMSS, Guadalajara, Jalisco, México; 4) Facultad de Medicina, Universidad de Coahuila, Coahuila, México.

Hyalinosis is a rare autosomal recessive inherited entity (OMIM 236490). There are 3 types that includes infantile (IH), juvenile hyalinosis (JH) forms and Urbach-Wiethe disease (hyalinosis cutis et mucosae). Infantile Systemic Hyalinosis (ISH) has been proposed as a disorder of collagen type VI characterized by thickening and nodularity of the skin, joint contractures, osteoporosis, and gingival enlargement. Failure to thrive that starts from the neonatal period, with death usually occurring before the third year of life, mainly before the second year. There is associated severe persistent diarrhea, wasting and recurrent severe respiratory infections. We report the case of a Mexican one-year-old boy. The propositus, the fourth child of healthy unrelated parents, was born after a gestation of 40 weeks. Birth weight was 3.6 Kg and length was 40 cm. Since first months of life he presented stiff skin and painful joint contractures, as well as all the typical features above mentioned. Increased excretion of urinary glycosaminoglycans was found in our patient (dermatan sulfate, heparan sulfate and chondroitin-4-sulfate) He had anemia microcytic hypochromic. The karyotype in the patient was 46,XY,inv (22) (p13;q13). The karyotype in the father was 46,XY,inv(22)(p12;q12). Increased excretion of urinary glycosaminoglycans has been reported in some cases, a data inconsistent in other reported cases. It is important point out the data of the karyotype witch could suggest a possible localization of a ISH gene.

**Genetic Investigation of association between presence of LHON primary point mutations and occurrence of MS phenotype in Iranian patients.** *F. Sharifpanah<sup>1</sup>, M. Houshmand<sup>1</sup>, M.H. Sanati<sup>1</sup>, Sh. Lavasani<sup>1</sup>, I. Rashedi<sup>2</sup>, J. Lotfi<sup>2</sup>.*  
1) Medical Genetics Unit, National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran; 2) Shariati Hospital, Tehran, Iran.

The hypothesis that mitochondrial genes may be implicated in susceptibility to multiple sclerosis (MS) is supported by an increasing number of case reports on Leber Hereditary Optic Neuropathy (LHON) associated mitochondrial DNA (mtDNA) point mutations in patients with MS. A number of mtDNA mutations with primary pathogenic significance for LHON, a maternally inherited disease causing severe bilateral visual loss predominantly in young men, have been detected in patients with a MS-like phenotype. To evaluate the link between MS and LHON primary point mutations, we investigated 31 unrelated Iranian clinically definite MS patients (23 females and 8 males) with optic nerve involvement, 3 of them with severe bilateral visual loss, as well as 25 patients (16 females and 9 males) without involvement of optic nerve as controls, for the presence of LHON mitochondrial mutations at nucleotide positions (np) 11778, 3460 and 14484 by mutation specific polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) and single strand conformation polymorphism (SSCP). Our results suggest that there is no association between Iranian patients with MS and mtDNA point mutations at np 11778, 3460 and 14484.

**Human Growth Hormone Treatment of Amyoplasia.** *C. Techakittiroj, E. Felner, D. Africk, H. Neitzschman, J. Thoene.* Hayward Genetics Center, Tulane University School of Medicine, Departments of Pediatrics, Neurology, and Radiology, New Orleans, LA.

Amyoplasia is a severe birth defect of unknown pathogenesis. A 38 week 3 kg female fit the diagnostic criteria: no spontaneous movement at birth; Apgar scores of 1, 4 and 6 at 1,5 and 10 minutes; elbows limited to extension; syndactyly of right 3rd and 4th digits; normal height; midline facial hemangioma; scaphycephaly; dimples over bony prominences; fractured right femur; hip flexion; fingers and toes locked in extension; equinovarus; hyperlordosis and hirsutism. An initial CNS MRI was normal. A total body MRI disclosed absence of limb muscle and "thready" psoas, paraspinous and gluteal muscles. An upper arm biopsy showed no muscle grossly or on Gomori-trichrome stain. EMG and NCV were non-conclusive. She began monthly HGH injections (depot Nutropin 1.5 mg/kg) at 21 months of age. After 4 months of therapy she has some voluntary finger grasp, and moderate head control. She has an ongoing vocabulary of 20 words and is intellectually normal for age at this time. Her urinary creatinine has varied from 1.9 g/kg body weight/8 prior to GH therapy to 2.3 after 4 months of treatment. A repeat MRI demonstrated increase in the volume and signal of the paraspinous, psoas and pelvic muscles with increase in gluteal muscle development as well. There are also minimal strands of muscle signal within the fat of the proximal extremities. The natural history of most (85%) patients with this condition is to show significant gains in strength and to ambulate unaided by age 5 years (Sells JM, Jaffe KM, and Hall JG, *Pediatr*, 97, 225-231, 1996). A positive effect of growth hormone therefore cannot be inferred in this patient. Follow up MRI studies of limb and axial musculature in this patient will permit better delineation of acquisition of muscle tissue in this condition.

**Clinical and genetic heterogeneity in familial dominant muscular dystrophy with pathological fractures. S.**

*Mehta<sup>1</sup>, G.D.J. Watts<sup>1</sup>, J. Wymer<sup>1</sup>, S.J. Hamilton<sup>1</sup>, B. McGillivray<sup>2</sup>, V.E. Kimonis<sup>1</sup>.* 1) Division of Genetics and Metabolism, Childrens Hospital, Harvard Medical School, 300 Longwood Avenue, Fegan 5, Boston, MA 02115; 2) Provincial Medical Genetics Programme, Children's and Women's Health Centre of British Columbia C234, 4500 Oak Street, Vancouver, British Columbia, Canada.

We have previously reported the unusual combination of autosomal dominant Paget disease of the bone (PDB) with a limb-girdle/inclusion body myopathy associated with dementia (IBMPFD). This disorder has been mapped to chromosome 9p22.3-q12 (Kovach et al, 2001). This present study describes an unrelated family previously reported by Henry et al. (1958) with an autosomal dominant progressive limb girdle muscular dystrophy, early onset of fracturing with poor healing, osteomyelitis and limb amputations. Some members also have herniae, premature greying, and a Von Willebrand type haematological picture.

Informed consent was obtained. Clinical, biochemical and radiological features have been documented in 9 males and 4 females. The average age of onset of the limb girdle myopathy was 29 y. occurring in 77% of affected individuals. The average age of onset of fractures was 20 y. occurring in 69% of affected individuals. Biochemical analysis showed a low normal alkaline phosphatase of 60 U/L (Normal 30-120) and high creatinine phosphokinase of 236.5 U/L (Normal 0-150). Radiographs show coarse trabeculation, sclerosis, cortical thickening, and narrowing of the medullary cavity. They were not suggestive of Pagets however there were features of fibrous dysplasia. Muscle biopsies and electromyograms showed myopathic changes. Nerve conduction studies were normal. Collagen studies were normal. Linkage analysis was undertaken for markers for the chromosome 9p22.3-q12 locus. This family does not map to this locus suggestive of a unique locus.

Screening of candidate genes and genome wide genotyping is in progress to identify the gene involved in the pathogenesis of this unique disorder. We report a unique autosomal dominant disorder with the combination of muscular dystrophy and bone fragility.

**Investigation of mtDNA common deletion in a patient affected with HIBM.** *S. Joughehdoust<sup>1</sup>, Y. Shafeghati<sup>1</sup>, M. Ataii<sup>1</sup>, D. Darvish<sup>2</sup>, M. Houshmand<sup>1</sup>.* 1) NRCGEB, Tehran, Iran; 2) American Board of Internal Medicine, USA.

Hereditary Inclusion Body Myositis is a chronic progressive muscle disorder that is prevalent in Jewish descendants from Iran and other middle east countries. There are several types of this disorder but HIBM(IBM2) have autosomal recessive pattern. 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 was 24 years old girl that suffered from muscle weakness. Positive findings in her physical exam were mild weakness in limbs muscles. The quadriceps was normal. EMG showed myopathic feature and muscle biopsy was compatible with muscular atrophy, neurogenic. All of the clinical and laboratory findings were typical for HIBM. The patient is from an unconsanguineous marriage and her grand parents were from Iranian Jewish ancestors. In our lab we have analyzed a mutation in the 3'UTR region of CNTFR mRNA gene, this gene is a 305 bp long. The method was PCR/RFLP(Res/ENZ:MspI). She was Homozygous for this mutation. MtDNA myopathies and HIBM share some similarities in their clinical findings and the base of both disorder is lack of ATP synthesis and IBM is another type of myositis that its relation with mtDNA deletions has been evaluated in recent reports. We checked the patient for mtDNA common deletion but no mutation was detected. Further investigation will confirm the relation between mtDNA mutations and HIBM.

**Partial deletion of the NSD1 gene in a patient with Sotos syndrome.** *V. Drouin-Garraud<sup>1</sup>, P. Saugier-veber<sup>1</sup>, C. Lecointre<sup>2</sup>, C. Hervé<sup>3</sup>, S. Fehrenbach<sup>1</sup>, T. Frebourg<sup>1</sup>.* 1) Department of Genetics, Rouen University Hospital, France; 2) Department of Paediatrics, Rouen University Hospital, France; 3) Department of Neonatology, Rouen University Hospital, France.

Sotos syndrome is an overgrowth syndrome characterized by pre- and postnatal overgrowth, macrocephaly, advanced bone age, and typical facial features. Recently, large 5q35 microdeletions detected by FISH, encompassing the NSD1 gene, and intragenic NSD1 mutations were reported in Sotos patients. Nevertheless, despite careful clinical screening, mutations or deletions have been detected in only 76 % of Sotos patients. Here we report on the case of a young girl affected with Sotos syndrome associated with athyreosis and harboring a partial deletion of the NSD1 gene. The patient is the second child of unrelated parents. Neonatal screening revealed hypothyroidism secondary to athyreosis which was treated with L-thyroxin. She developed West syndrome at 5 months of age. At 8 years, overgrowth and macrocephaly were obvious, with height and head circumference above the 98th centile. She had characteristic dysmorphic facial features of Sotos syndrome and mild mental retardation. Hands radiographs showed advanced bone age. R-banding lymphocyte karyotype was normal. We analyzed the NSD1 gene, using QMPSF (Quantitative Multiplex PCR of Short Fluorescent Fragments), a simple method for the detection of heterozygous rearrangements which is based on the simultaneous amplification of short fluorescent fragments. In this patient, we identified a partial deletion of the NSD1 gene removing exon 23 but respecting exon 2. The FLJ21458 gene located 3.4 Mb telomeric from NSD1 was not deleted. This case report demonstrates that partial deletions of the NSD1 gene can arise and may account for some of the 24 % of cases in which no NSD1 mutation / deletion has been detected.

**The paternal age effect in Apert syndrome is due in part to the increased frequency of mutation in sperm in the general population and group-specific characteristics of Apert syndrome fathers.** *R.L. Glaser<sup>1</sup>, K.W. Broman<sup>1</sup>, R.L. Schulman<sup>1</sup>, B. Eskenazi<sup>2</sup>, A.J. Wyrobek<sup>3</sup>, E.W. Jabs<sup>1</sup>.* 1) Johns Hopkins Univ, Balto, MD; 2) Univ Calif. Berkeley, CA; 3) Lawrence Livermore National Lab, Livermore, CA.

An increased incidence of affected children with paternal age and the paternal origin of mutations have been observed for sporadic cases of several autosomal dominant disorders. Mutations in these disorders are all single nucleotide changes thought to result from DNA copy errors during spermatogonial stem cell replication. One such disorder is Apert syndrome (AS) which is characterized by craniosynostosis and syndactyly of the hands and feet and occurs in 1/65,000 to 160,000 live births. As the incidence of sporadic AS births increases exponentially with paternal age, we hypothesized that the frequency of AS mutations in sperm would also increase. To determine the frequency of two recurrent FGFR2 mutations, 755C>G and 758C>G, accounting for 99% of all cases of AS, we developed allele-specific peptide nucleic acid-PCR assays sensitive enough to detect 1/50,000 mutant sperm. Using this method to analyze sperm DNA from 148 men ages 21-80 years we showed that the number of sperm with either FGFR2 mutation increased in the oldest age groups among men who did not have an AS child ( $p=0.008$ ). These older men were also more likely to have both mutations in their sperm. However, this age-related increase in mutation frequency was not sufficient to explain the AS birth frequency. In contrast, a significantly increased mutation frequency was observed in men who had AS children ( $p=0.002$ ), most of whom were younger than 45 years old. These younger fathers had sperm mutation frequencies that resembled those of men older than 60 years who had not fathered AS children. Therefore, the paternal age effect may be due to a higher number of mutant sperm and an earlier occurrence of mutations in the subset of men ascertained because they had a child with AS. No age-related increase in the frequency of these mutations was observed in leukocytes. Selection and/or quality control mechanisms, including DNA repair and apoptosis, may contribute to the cell type differences in mutation frequency.

**Mosaicism for two large, different MECP2 gene deletions in a classic RTT female, apparently caused by the increased size of an original mutation.** *L. Giunti<sup>1</sup>, S. Guarducci<sup>1</sup>, U. Ricci<sup>1</sup>, C. Bravaccio<sup>2</sup>, M.L. Giovannucci Uzielli<sup>1</sup>.* 1) Paediatrics - Genetics Unit, University of Florence, Florence, Florence, Italy; 2) Children Neuropsychiatry Unit, II University of Naples, Italy.

Rett syndrome (RTT) is one of the most frequent neurodevelopmental disorders occurring almost exclusively in females. In 1999, RTT was reported to be caused by mutations in the gene encoding methyl-CpG-binding protein 2 (MECP2), located at chromosome Xq28. RTT had been thought to be an X-linked dominant condition leading to prenatal death in hemizygous males. Since the discovery of mutations of the MECP2 gene as the cause of Rett syndrome, MECP2 mutations have, however, also been reported in males. It is now clear that in males with classical Rett syndrome the mutation of MECP2 arises as somatic mosaicism or in presence of an extra X chromosome. Detection rate of MECP2 gene in classical cases of RTT females is 75-85%. Approximately 70% of sporadic RTT patients have missense or truncated mutations. Over 95% of mutations are de novo. About 95% of the missense, nonsense, and one or two base pair insertion or deletion mutations occur in the 3 half part of exon 3 and the 5 half of exon 4 of MECP2 gene. The gross deletions were in the 3 half of exon 4. Few cases of multiple deletions of MECP2 gene were reported. Two descriptions of somatic mosaicisms are published. In a group of 160 RTT patients positive for MECP2 mutation, by direct sequencing analysis, we found more than 15 large deletions with all breakpoints within a 3 185-bp region of MECP2 exon 4. In all cases, the gross deletion was single and apparently homogeneous. In a classic RTT female we demonstrated the absence of the entire exons 3 and 4 by using the MLPA technique (Multiplex Ligation-dependent Probe Amplification). We report here a new case of classic RTT female with a very large deletion of MECP2 genes exon 4, with three alleles. The wild allele, and two mutated alleles with different sized deletion: one with a 412 bp deletion and a L408X stop codon, a second one with an intermediate deletion starting at the same nucleotide 22611. Further studies are in progress to better characterize the origin of the mutations.

Program Nr: 723 from 2003 ASHG Annual Meeting

**Deletion of exons 3 and 4 of MECP2 gene, revealed by MLPA technique, in a RTT Italian girl with a classic phenotype.** *M.L. Giovannucci Uzielli, L. Giunti, S. Guarducci, U. Ricci, I. Sani, E. Lapi, E. Andreucci, C. Bacci, B. Toschi, M. Ottaviani.* Paediatrics, Genetics Unit, Univ Florence, Florence, Italy.

A novel mutation detection approach, called Multiplex Ligation-Dependent Probe Amplification (MLPA), was able to reveal a large genomic deletion not identified by direct sequencing. Federica is an Italian girl with classical Rett syndrome. The direct sequencing analysis of the entire coding region did not reveal any mutation of MECP2 gene. We identified a heterozygous C>A transversion at 375 (1125) in the mother, while the father showed a wild normal allele. The daughter showed only the maternal allele with the C>A substitution, and we placed the hypothesis of a lack of entire exons as cause of RTT phenotype. We used the MLPA (Multiplex Ligation-Dependent Probe Amplification Technique), a quantitative multiplex PCR approach, to determine the relative copy number of each MECP2 gene exons. We were able to confirm the deletion of entire exons 3 and 4. This method reveals alterations of genomic DNA that might escape detection using conventional diagnostic techniques. It is especially useful in RTT mutational studies, because the direct sequencing of MECP2 gene does not reveal any apparent MECP2 mutation in 15-25% of Classic and non-classic RTT patients.

**Uncommon FBN1 mutation in Marfan syndrome family with severe ectopia lentis.** *M. Manning<sup>1</sup>, J. Hyland<sup>2</sup>, A. Kwan<sup>1</sup>, D. Liang<sup>1</sup>, L. Hudgins<sup>1</sup>.* 1) Stanford University, Stanford, CA; 2) Tulane University Health Sciences Center, New Orleans, LA.

Marfan syndrome (MFS) is a multisystem disorder involving the skeletal, cardiovascular and ocular systems. Mutations in the gene fibrillin-1 (FBN1) have been identified as causing MFS as well as other related disorders, such as ectopia lentis (EL) and familial aortic aneurysm. There have been 337 mutations in FBN1 located throughout the gene. Due to clinical variability within the condition, work is ongoing to determine genotype/phenotype correlation in order to provide appropriate management.

We present a family in which the 8 yo male proband was originally referred to rule out a suspected diagnosis of MFS. Bilateral EL was noted in 3 family members, with no other Ghent diagnostic criteria present for the diagnosis of MFS. Familial EL was the presumed diagnosis. FBN1 mutation analysis revealed a 364 C>T in exon 4 resulting in a cysteine for arginine substitution at aa 122, a change that results in an extra cysteine in the EGF#2 domain. The mutation has been reported on 4 other occasions and is considered disease causing. Published clinical descriptions are brief, however all affected individuals had major ocular manifestations (EL) with only minor skeletal system involvement. One family demonstrated late onset minor cardiovascular findings. Our 3 subjects were also noted to have a second, previously unreported mutation at aa 124 in which valine was substituted for methionine. This sequence variation results in 2 aa substitutions in *cis* separated by only one other aa. The effect on protein structure of this domain has not been determined. Subsequent echocardiograms in 2 of our subjects revealed aortic root dilation for their body mass index.

The findings in our family suggest that FBN1 mutation analysis is indicated in families with primarily EL, as results may provide additional criteria to make a diagnosis of MFS. In particular, individuals with the 364 C>T mutation may be at high risk for cardiovascular involvement, as well as EL, and should be followed expectantly.

**Noonan syndrome with café-au-lait spots and LEOPARD syndrome: evidence for genetic heterogeneity.** *A.M. Nyström, G. Annerén, M.L. Bondeson.* Dept of Genetics & Pathology, Uppsala University, Rudbeck Laboratory, Sweden.

Noonan syndrome (NS) is an autosomal dominant disorder characterised by facial dysmorphism, congenital heart defects and short stature. Among associated findings are skin manifestations such as café-au-lait spots and lentigines. Several clinical features of NS overlap with other syndromes, e.g. LEOPARD syndrome and Neurofibromatosis type I. Missense mutations in the *PTPN11* gene at 12q24.1 are associated with NS in about 50% of the patients. Recently, mutations in the *PTPN11* have also been reported to be associated with LEOPARD syndrome. Mutations in the *NF1* gene at 17q11.2 are associated with Neurofibromatosis type I and *NF1* mutations have also been identified in some patients with Neurofibromatosis-Noonan syndrome (NFNS). Here, we report the findings of mutation screening and linkage analysis of the *PTPN11* and *NF1* genes in two families affected by NS and/or café-au-lait spots and one family with the LEOPARD syndrome. Linkage to the *PTPN11* gene was found in a two-generation family affected by the LEOPARD syndrome. The coding region of the *PTPN11* gene was screened by dHPLC followed by direct sequencing in the probands. The result revealed a missense mutation 836A>G; Y279C segregating with the disorder in the family. The Y279C mutation has previously been described in LEOPARD patients, suggesting that it may represent a recurrent mutation in the *PTPN11* gene for the LEOPARD syndrome. A mutation in the *PTPN11* gene (F285L; 853T>C) was also identified in a proband with NS and café-au-lait spots. In this family, the sister of the proband and the father presented with short statures and café-au-lait spots but with no other clinical manifestations of NS. However, mutation analysis revealed that they were not carriers of the F285L mutation, suggesting that the café-au-lait spots and NS in this family are caused by distinct genetic entities. Linkage analysis in a three-generation family diagnosed with NS and café-au-lait spots revealed that the disorder in this family is neither linked to the *NF1*- nor to the *PTPN11* locus. Our result thus suggest that NS with café-au-lait spots is genetically heterogeneous and may even present as different genetic entities.

**Identification of two novel mutations of IRF6 in Korean families affected with Van der Woude syndrome. J.-Y.**

*Park*<sup>1</sup>, *H.-W. Yoo*<sup>1,2</sup>, *Y. Kim*<sup>1</sup>. 1) Genome Research Center for Birth Defects and Genetic Disorders, Ulsan University of College of Medicine, Asan Medical Center, 388-1, Pungnap-Dong, Songpa-Gu, Seoul 138-736, Korea; 2) Department of Pediatrics, Ulsan University of College of Medicine, Asan Medical Center, 388-1, Pungnap-Dong, Songpa-Gu, Seoul 138-736, Korea.

Van der Woude syndrome (VWS; OMIM 119300) is an autosomal dominant disorder with a high degree of penetrance. Clinical characteristics of VWS include cleft lip with or without cleft palate (CL/P), bilateral lip pits, isolated cleft palate, and hypodontia. Recently, the interferon regulatory factor 6 gene (*IRF6*) has been identified as the gene mutated in VWS. *IRF6* belongs to a family of nine interferon regulatory factors, all of which share a highly-conserved DNA-binding domain in the N-terminus. Through the DNA-binding domain, IRFs elicit their effects of transcription activation on the target genes. *IRF6* also contains a putative Smad-binding domain in the C-terminus. The Smads proteins are a family of transcription factors known to transduce signals of the TGF- superfamily. Using PCR-direct sequencing of the exonic regions of *IRF6*, we found two novel mutations of *IRF6*, 399delC and G74C, from two unrelated Korean families with VWS. The 399delC mutation was identified from a family showing complete cleft lip and palate with a lower lip pit in an affected daughter. Her father, carrying the same mutation, showed bifid uvula with a pit on his lower lip. This mutation caused a frame-shift at pro133 and resulted in a premature termination at codon 165, abolishing the C-terminal region of *IRF6*. The second mutation, G74C, was detected from an affected son and his mother, both suffered from bilateral cleft lip and palate with pits on the lower lip. The G74C mutation substituted an alanine for a glycine at codon 25 in the DNA-binding domain. Both mutations are presumably expected to disturb the transcription regulatory function of *IRF6*. Our findings further confirm that haploinsufficiency of *IRF6* is responsible for the phenotypic features of VWS. The newly identified mutations will be useful for further understanding of the essential roles of *IRF6* in morphogenesis of the palate and lip.

**Candidate syndromic genes in nonsyndromic cleft lip and palate.** *N. Prescott, C. Garcia-Rodriguez, M. Lees, RM. Winter.* Clinical & Molecular Genetics, Inst Child Health, London, United Kingdom.

Nonsyndromic Cleft lip with or without cleft palate (CL/P) is an epithelial fusion defect that occurs during the first few weeks of pregnancy. This congenital anomaly is influenced by genetic factors as demonstrated by an elevated sibling relative risk of 30-40. Despite, the underlying genetic etiology of CL/P is likely to be complex/ multifactorial and influenced by environmental factors. Recently a mutation in the poliovirus receptor-like 1 (PVRL1) gene has been found in a rare syndromic form of cleft lip with ectodermal dysplasia (CLPED1) the same mutation has a high frequency of carriers in sporadic cases of NSCL/P from a geographically adjacent region in Venezuela (Sozen et al., 2001). It seems very likely that there will be examples where allelic changes in other genes underlying syndromic forms of CL/P will cause nonsyndromic CL/P. We can use this hypothesis to predict potential candidate genes for nonsyndromic CL/P. We have used 96 sporadic cases with CL/P and two dominant families with CL/P and associated anomalies to screen the candidate genes PVRL1, SKI oncogene, IRF6 and EDN1 by fluorescent heteroduplex analysis to identify variations that may influence risk for nonsyndromic CL/P. We have identified a number of individuals giving heteroduplexes (HD) that may represent novel variants in SKI, IRF6 and EDN1. DNA sequencing of HDs in EDN1 has revealed a novel variation in exon 5 in one affected male and his unaffected father. Further sequencing result on the remaining genes will be presented.

**CONFIRMATION OF GENETIC HETEROGENEITY IN SUBCORTICAL BAND HETEROTOPIA.** *F.R. Torres<sup>1</sup>, M.A. Montenegro<sup>2</sup>, M.M. Guerreiro<sup>2</sup>, F. Cendes<sup>2</sup>, I. Lopes-Cendes<sup>1</sup>*. 1) Department of Medical Genetics, UNICAMP, Campinas, Brazil; 2) Department of Neurology, UNICAMP, Campinas, Brazil.

**Purpose:** Subcortical band heterotopia (SBH) is a neuronal migration disorder causing developmental delay and epilepsy. Female patients with SBH, who have a malformation gradient with anterior predominance, usually have mutations in the *DCX* gene. In addition, there are few male patients with SBH and mutation in the *LIS1* gene. The objective of this study is to describe neuroimaging and molecular findings of a cohort of patients with SBH. **Methods:** All patients included in this study had high resolution MRI scans. We searched for mutations in the *DCX* and *LIS1* genes. In addition, a total of 50 unrelated normal control subjects were also genotyped. PCR reactions were performed with primers designed to amplify the coding region and intron/exon boundaries of *DCX* and *LIS1*. PCR samples were analyzed by the single-strand conformation polymorphism method. The nucleotide sequence of all fragments showing an altered band shift were subsequently determined using the Big Dye Terminator Sequencing kit for megaBACE1000. **Results:** We have studied a total of 6 patients with SBH and divided them into 2 groups, according to neuroimaging criteria, in: typical and atypical SBH. We found 4 patients (1 male and 3 female) with the typical SBH, which is usually linked to *DCX* mutations; as well as, 2 female patients with atypical findings, such as SBH with posterior predominance and the association SBH-pachygyria. None of the patients, including those with typical phenotypes, showed mutation in *DCX* and *LIS1*. We only found a normal variant (1805CT) in the 3 untranslated region of the *LIS1* gene in 4 patients, which was also present in 9 control subjects. **Conclusion:** Our results confirm the presence of genetic heterogeneity in SBH and points to the possibility of mutations in at least a third, still undetermined, gene. In addition, we corroborate previous findings indicating that patients with atypical SBH do not have mutations in *DCX*, and that the occurrence of *DCX* and *LIS1* mutations in males with SBH is a very rare event. Supported by CAPES and FAPESP.

Program Nr: 729 from 2003 ASHG Annual Meeting

**Congenital temporomandibular joint ankylosis (CTMJA): clinical characterization of 3 unrelated affected.**  
*V.L.G.S.L. Lopes, D.V.L. Luquetti.* Medical Genetics Dept, State Univ Campinas, Campinas, Sao Paulo, Brazil.

Congenital temporomandibular joint ankylosis (CTMJA) is a rare feature characterized by facial disfigurement, significant reduction in mouth opening, difficulties in feeding and breathing. In some cases, secondary cor pulmonale can be detected. Etiology of this disorder seems to be heterogeneous; traumatic form, in which forceps delivery is considered an important factor, as well as infection diseases should be mentioned. It is often diagnosed around sixth month of life, in view of some joint mobility present at birth, before the ossification of the cranial sutures. At least, a genetic form has been suggested based upon a case report describing siblings of different sexes with no history of parental consanguinity or description of associated anomalies. However, there is few information about genetics aspects of this condition. We report 3 unrelated patients, 2 males and 1 female, presenting CTMJA. One of the male presented developmental delay and the girl had congenital general hypotonia. Clinical findings suggested different etiologies among them. Therefore, the dysmorphological characterization of these cases, including the clinical evolution, could be helpful in recognize and delineate this condition, favoring the clinical follow up of affected individuals.e-mail: cranface@fcm.unicamp.b.

**Long term outcome of feeding problems in Noonan syndrome.** *A.C. Shaw, M. Sharland, A.H. Crosby, K. Kalidas, G. Sivapalan, S. Jeffery, M.A. Patton.* Medical Genetics, St George's Hospital Medical School, London, UK.

Feeding problems are a common finding in infants with Noonan syndrome (NS). There is wide variation ranging from a weak suck and occasional vomiting to severe and protracted difficulties requiring feeding via nasogastric (NG) tube. The feeding histories of 144 infants with NS were published in 1992, and 117 (81%) of this cohort have now been followed up for in excess of 10 years.

Severity was graded on a scale of 0 to 3 where 0 indicates no feeding problems (24%), 1 for weak suck (14%), 2 for additional frequent and forceful vomiting (38%) and 3 for when NG tube feeding was required for over 2 weeks (24%). Analysis of this cohort reveals that for each grade of severity the proportion of patients with *PTPN11* mutations is 30%; 36%; 39% and 39% respectively. The mean age of onset of speaking in 2 word phrases for each grade is 27; 26; 36 and 40 months respectively ( $p=0.036$ ). The proportion of patients attending special school for each grade is 16%; 11%; 40% and 60% ( $p=0.005$ ). Educational attainment was better in those without feeding problems with 40% going on to higher education whereas the highest achievement reached in the severe group were GCSE exams (national exam for 16 year olds). The median childhood weight centile for each grade was 10<sup>th</sup>, 10<sup>th</sup>, 3<sup>rd</sup> and 2<sup>nd</sup> ( $p=0.08$ ) while the median childhood height centile was 2<sup>nd</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 0.4<sup>th</sup> ( $p=0.5$ ).

In conclusion, severe feeding difficulties in infancy are a predictor of marked speech delay, special educational need and poor educational attainment in later life. Such feeding difficulties may be an early manifestation of neurological impairment and it is of note that there is little correlation with growth restriction, suggesting that the poor nutritional intake in infancy is not the major cause of developmental delay.

**Evaluation of genotype-phenotype correlations for vestibular schwannoma growth rates in neurofibromatosis 2**

**(NF2).** *D.M. Parry*<sup>1</sup>, *A. Jackson*<sup>2</sup>, *A.J. Wallace*<sup>3</sup>, *L. Kluwe*<sup>4</sup>, *V.-F. Mautner*<sup>5</sup>, *R.T. Ramsden*<sup>6</sup>, *D.G.R. Evans*<sup>3</sup>, *M.E.*

*Basler*<sup>7</sup>. 1) Genetic Epidemiology Branch, National Cancer Institute, Bethesda, MD, USA; 2) Division of Imaging Science and Biomedical Engineering, University of Manchester, Manchester, UK; 3) Department of Medical Genetics, St. Mary's Hospital, Manchester, UK; 4) Department of Neurosurgery, University Hospital Eppendorf, Hamburg, Germany; 5) Department of Neurology, Klinikum Nord Ochsensoll, Hamburg, Germany; 6) Department of Otolaryngology, Manchester Royal Infirmary, Manchester, UK; 7) Los Angeles, CA, USA.

We reported that age at onset of symptoms or age at diagnosis was an important determinant of vestibular schwannoma (VS) growth rates in NF2, although growth rates were highly variable, even among multiple patients of similar ages in the same family (J Neurosurg 2002;96:217-22 and 223-28). In this study, we evaluated genotype-phenotype correlations for VS growth rates in NF2. We retrospectively measured serial VS volumes from gadolinium-enhanced MRI scans in 80 patients from 62 NF2 families; the median length of observation was four years. SSCP was used to screen for constitutional *NF2* mutations. Linear regression was used to compare VS growth rates of patients with splice-site mutations, missense mutations, in-frame deletions, or unidentified mutations to growth rates in patients with nonsense or frameshift mutations. VS growth rates tended to decrease exponentially with increasing age at initial VS volume measurement ( $r^2 = 0.26$ ,  $P < .001$ ), and there was high intra-familial variability in VS growth rates. In three families with missense mutations, the intrafamilial variability in VS growth rates among patients of similar ages was 1.3-fold, 1.8-fold, and 31.2-fold. After accounting for age at initial VS volume measurement, VS growth rates were not significantly lower in people with any type of non-truncating mutation, or in people with unidentified mutations, than in people with nonsense or frameshift mutations. These results suggest that, in addition to age, as-yet-unknown factors are important determinants of VS growth rates in NF2 (e.g., growth factors, modifying genes, or complex interactions).

**Genome wide screen for cryptic deletion in case-parent trios with CHARGE Syndrome and development of an Expected Deletion Detection (EDD) metric.** *J. Belmont<sup>1,2</sup>, L.M. Molinari<sup>1</sup>, S.D. Fernbach<sup>1</sup>, A. Safiullah<sup>1</sup>, K. McBride<sup>1</sup>, S.R. Lalani<sup>1</sup>.* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept of Pediatrics; Baylor College of Medicine.

CHARGE syndrome is characterized by congenital heart disease, coloboma of the eye, choanal atresia, retardation of mental and somatic development, genital abnormalities, ear abnormalities and/or deafness. Because of the complex phenotype, overlap with velocardiofacial syndrome, and the relatively high frequency, one hypothetical etiological mechanism is a cryptic deletion. We undertook a high-resolution genome wide screen using 9 CHARGE case-parent trios testing for Mendelian inconsistency with 3258 single nucleotide polymorphism (SNP) markers. The average marker spacing was approximately 880kb. Across all markers, Mendelian inconsistencies could be used to estimate the observed and actual genotype error rate. We developed a novel analytical method to estimate the proportion, Expected Deletion Detection (EDD), of deletions of a specified size that might be detected in the experimental sample. The method takes into account the intermarker distances over floating windows of 3 markers each. It allows estimation of possible consequences of the suspected deletion covering 1, 2, or 3 markers depending on its size and position in the window. The model also takes into account the incomplete information provided by diallelic SNPs and the effect of allele frequencies on the proportion of undetected deletions. The model assumes a uniform deletion i.e. EDD increases with number of trios, but in order to accommodate the underlying genotype error rate a threshold of 2 inconsistencies per marker was employed. In this way single chromosome maps were generated giving an overview of the regions unlikely to bear a deletion of at least 2 Mb. A single marker gave genotypes violating Mendelian inheritance in 4 pedigrees. Followup FISH and genotypes of adjacent markers failed to confirm the presence of a deletion, however.

**A novel polymorphic site at the mutation hot-spot(TM VI)of the luteinizing hormone receptor in a patient with familial male-limited precocious puberty.** *M.YK. Leung<sup>1</sup>, V. Baxendale<sup>1</sup>, E. Leschek<sup>2</sup>, S.M. Wu<sup>1</sup>, K. Fichman<sup>3</sup>, W.Y. Chan<sup>1,4</sup>, O.M. Rennert<sup>1</sup>.* 1) LCG, NICHD, NIH, Bethesda, MD; 2) Developmental Endocrinology Branch, NICHD, NIH, Bethesda, MD; 3) Department of Pediatrics, Kaiser Permanente, San Rafael, CA; 4) Departments of Pediatrics and Cell Biology, Georgetown University, Washington, DC.

Activating mutation of the human luteinizing hormone receptor (hLHR) is the cause of familial male-limited precocious puberty (FMPP). The symptom is characterized by LH independent elevated testosterone level and the early onset of puberty in boys. There are at least 16 such hLHR mutations reported, which affect 13 a. a. residues. The mutations are located in transmembrane helices (TM) I, II, III, V, and VI and cytoplasmic loop 3. Eight of the mutations, are located in TMVI. TMVI is, thus, the hot-spot for activating mutations. A 4/12 year old patient with enlarged penis, pubic hair development, tall stature (39 inches), prepubertal LH levels but elevated testosterone was referred to our laboratory for molecular diagnosis of FMPP. Congenital adrenal hyperplasia in the patient had been excluded. Analysis of hLHR exon 11 by nucleotide sequencing revealed the presence of a heterozygous A1738G transversion mutation in TMVI. The mutation changed threonine-580 to alanine. The mutant hLHR cDNA was transiently expressed in HEK293 cells to study basal cAMP production and the response to hCG stimulation. The mutated receptor was unable to trigger a cAMP level higher than that of wild-type hLHR in the absence of hCG. The response of the mutated and wild-type hLHR to hCG stimulation was comparable. Thus, although the mutation is located in the hot-spot and caused considerable change in the characteristic of the affected a. a. residue, no increased activity of the mutant hLHR was observed. Therefore, it is unclear whether this patients precocious puberty can be ascribed to this mutation in hLHR. These observations suggest that caution should be taken in the interpretation of results of molecular analysis in the diagnosis of FMPP and emphasized the importance of in vitro expression studies to confirm the biological consequences of detected mutations.

**Description of the Pulmonary Function Tests in 13 Spondylothoracic Dysplasia Patients.** *A. Cornier<sup>1</sup>, A.A. Reis<sup>1</sup>, J.M. Massanet<sup>1</sup>, J.R. Acevedo<sup>3</sup>, S.E. Carlo<sup>1</sup>, V. Franceschini<sup>1</sup>, H. Rivera<sup>1</sup>, N. Ramirez<sup>2</sup>.* 1) Dept Biochemistry, Ponce Sch Medicine, Ponce, PR; 2) Departement of Orthopedics, Mayaguez Medical Center, PR; 3) Department of Nursing, Univ.PR-Arecibo, Arecibo, PR.

Spondylothoracic dysplasia (STD; MIM#277300) is an autosomal recessive disorder with high prevalence in the Puerto Rican population. Findings include segmentation and formation defects throughout cervical, thoracic and lumbar spine such as hemivertebrae, block vertebrae, and unsegmented bars with fusion of the ribs at the costo-vertebral junction (crab-like chest configuration). Pulmonary hypertension has also been described. It was generally regarded as a lethal condition in the neonatal period. However, we have prospectively followed a series of 13 patients, of which 6 were males and 7 were females, ranging from 7 to 49 years old, because the minimum age required for pulmonary function testing is 7 years, in our genetics clinic. Pulmonary function tests were performed by a respiratory care technician and supervised by a respiratory medicine specialist to all patients. The pulmonary function tests showed a severe restrictive pattern with an average FEV1/FVC ratio of 0.9. The average measurements were: FVC: 27.1 %, FEV1: 27.7 %, PEF: 43.0 %, of the predicted values for the group. Even though these values tend to be slightly higher in the female population (FVC: 30.1 %, FEV1: 28.5 %, PEF: 49.1 %) than in the male population (FVC: 23.8 %, FEV1: 25.4 %, PEF: 35.8 %), statistical analysis has not shown the differences to be significant (p values of 0.631 for FVC, 0.359 for FEV1, and 0.394 for PEF). This restrictive pattern may be due to the severely shortened thoracic vertebral column and the fusion of all the ribs at the costo-vertebral junction, which makes for a rigid chest wall that prevents the use of secondary muscles for breathing. Consequently, the diaphragm is pushed downwards and reaches the level of the iliac crests, resulting in a protuberant abdomen with increased pressure and propensity to hernias. Despite the harshly limited vital capacity, these patients do not show any impairment in their daily activities. They are able to work and exercise without major pulmonary problems.

**An obviously dysmorphic face maybe a predictor of cognitive impairment in Fetal Anticonvulsant Syndrome.** *U. Kini*<sup>1</sup>, *A. Fryer*<sup>2</sup>, *J. Clayton-Smith*<sup>1</sup>, *Liverpool Manchester Neurodevelopmental Study Group.* 1) Dept Clinical Genetics, St Mary's Hosp, Manchester, United Kingdom; 2) Dept Clinical Genetics, Alder Hey Children's Hospital, Liverpool, UK.

Prenatal exposure to antiepileptic drugs (AEDs) is known to cause dysmorphic facial features, major malformations and cognitive impairment in the child. Although the facial features and major malformations can be identified soon after birth, the cognitive impairment is not evident until later on in life. We determined the prevalence of dysmorphic features in children exposed prenatally to specific AEDs to investigate if the face of fetal anticonvulsant syndrome can be used as predictor of cognitive impairment in these children. **Methods:** In a retrospective study of 218 mothers recruited from regional epilepsy clinics in Manchester and Liverpool, we assessed 375 children exposed to different AEDs. Structured interviews, clinical examination and psychometric tests (WISC) were used to assess exposure, dysmorphic features and IQ (intelligence quotient). Blind assessment of photographs of children including those from early childhood were used to categorise children as mildly, moderately and severely affected based on dysmorphic features. **Results:** Dysmorphic features were more commonly seen in children exposed to valproate (VPA), with 44%; having moderate to severe dysmorphic features in contrast to those exposed to carbamazepine (9.2%) and the non-exposed, NE (2.2%). The mean full scale IQ in children exposed to VPA (87.2, CI 81.9 to 92.5) was lower than that in the NE group (89.5, CI 85.5 TO 93.4). The mean verbal IQ (VIQ) was significantly lower in the VPA group (83.61, CI 78.2 to 89.0) compared to NE (90.9, CI 87.2 to 94.6); of those with moderate to severe features had a very low IQ (less than 79). There was a significant correlation between VIQ and dysmorphic features in the VPA group (Spearman's rho = -0.436). **Conclusion:** The retrospective nature of our study precludes the ascertainment of absolute risks. Nonetheless, these results suggest that there may be a correlation between the degree of dysmorphism and the likelihood of significant cognitive impairment, particularly with VPA exposure.

**HOMOZYGOSITY FOR CAG MUTATION IN HUNTINGTON DISEASE IS ASSOCIATED WITH A MORE SEVERE CLINICAL COURSE.** *F. Squitieri*<sup>1</sup>, *C. Gellera*<sup>2</sup>, *M. Cannella*<sup>1</sup>, *C. Mariotti*<sup>2</sup>, *G. Cislighi*<sup>3</sup>, *D. Rubinsztein*<sup>4</sup>, *E. Almqvist*<sup>5</sup>, *D. Turner*<sup>6</sup>, *A. Bachoud Levi*<sup>7</sup>, *S. Simpson*<sup>8</sup>, *M. Delatycki*<sup>9</sup>, *V. Maglione*<sup>1</sup>, *M. Hayden*<sup>5</sup>, *S. Di Donato*<sup>2</sup>. 1) Neurogenetics, IRCCS NEUROMED, POZZILLI, IS, Italy; 2) Division of Biochemistry and Genetics, Neurological Institute IRCCS C. Besta, Milan, Italy; 3) Division of Neurology, Fornaroli Hospital, Magenta (MI), Italy; 4) Cambridge Institute for Medical Research, Addenbrookes Hospital, England; 5) Centre for Molecular Medicine and Therapeutics, Dept. of Medical Genetics, University of British Columbia, Vancouver, B.C., Canada; 6) Dept. of Haematology and Genetic Pathology, School of Medicine, Flinders University, Adelaide, Australia; 7) Service de Neurologie, Hpital Henri Mondor, Creteil, France; 8) Department of Medical Genetics, Grampian University Hospitals Trust, Aberdeen, Scotland; 9) Victorian Clinical Genetics Services, The Murdoch Institute, Royal Children's Hospital, Melbourne, Australia.

Huntington disease (HD) patients with two mutant alleles are very rare. In this multicenter study, we sought differences in the disease features between eight homozygotes and 75 heterozygotes for HD mutation. We compared clinical features of subjects homozygotes (age at onset, symptom presentation, disease severity, and disease progression) with those of a group of heterozygotes, longitudinally assessed. The age at onset of symptoms in the homozygote cases was within the range expected for heterozygotes with the same CAG repeat lengths (homozygotes 51.32.7 vs heterozygotes 48.63.9 years), whereas homozygotes had a more severe clinical course ( $p=0.0002$ ). The observation of a more rapid decline in motor, cognitive and behavioural symptoms in homozygotes was consistent with the extent of neurodegeneration as available at imaging in three patients and at the post-mortem neuropathological report in one case. Our analysis suggests that though homozygosity for the HD mutation does not lower the age at onset of symptoms it affects the phenotype and the rate of disease progression pointing to the possibility that the mechanisms underlying age at onset and disease progression in HD may differ.

**A clinical, molecular genetic and neuro-otological features in 140 cases of Spinocerebellar ataxia type 6 subjects.**

*H. Takahashi*<sup>1</sup>, *K. Ishikawa*<sup>1</sup>, *T. Tsutsumi*<sup>2</sup>, *K. Kitamura*<sup>2</sup>, *H. Mizusawa*<sup>1</sup>. 1) Department of Neurology and Neurological Science, Tokyo Medical and Dental University Graduate School of Medicine, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan; 2) Department of Audio-Vestibular Neuroscience.

[Purpose] Spinocerebellar ataxia type 6 (SCA6) is one of autosomal dominant cerebellar ataxias (ADCAs) and is caused by a small CAG-repeat expansion in the alpha1A (P/Q type)-subunit of voltage-gated calcium channel gene. Although the main clinical feature of SCA6 is slowly progressive ataxia, other additional symptoms are highly variable in past reports. The purpose of this report is to clarify the clinical, molecular genetic and neuro-otological characteristics of SCA 6 in a large number of Japanese cases with SCA 6. [Methods] We retrospectively analyzed clinical features of SCA6 patients molecularly diagnosed in our department. In total 140 patients were available of detailed clinical information and their clinical and molecular genetic findings were analyzed. Neuro-otological examinations were performed in 22 patients in the Department of Audio-Vestibular Neuroscience. [Results] Cerebellar signs were seen in nearly 100 % of SCA6 patients and nystagmus was present in 66.6 %. Some SCA6 patients had additional neurological features such as hyperreflexia (6.0%), tremor (8.6%), hyporeflexia (15.2%) and impaired vibration sense (4.3%), but these additional signs were variable, infrequent and, of mild degree. Neuro-otological findings suggested the close relationship between downbeat positioning nystagmus (DPN) and positioning vertigo. In caloric test visual suppression was frequently observed without caloric weakness. [Conclusion] Although the main clinical feature of SCA6 was slowly progressive ataxia, other additional symptoms including episodic symptoms were infrequent. Neuro-otological findings demonstrated purely cerebellar eye movement disorders, and positioning vertigo with DPN indicating SCA6 seemed to be related to the disturbance of cancellation of the vestibulo-ocular reflex.

**Genetic disturbances in Silver-Russell syndrome and significance for molecular genetic testing.** *T. Eggermann<sup>1</sup>, E. Meyer<sup>1</sup>, C. Obermann<sup>1</sup>, H. Wollmann<sup>2</sup>.* 1) Human Genetics, University Hospital, Aachen, Germany; 2) University Childrens Hospital, Tuebingen, Germany.

Silver-Russell syndrome (SRS) describes a uniform malformation syndrome characterised by severe intrauterine and postnatal growth retardation (IUGR/PGR), a small triangular face, clinodactyly V, relative macrocephaly and other less constant features. The syndrome is heterogeneous and various genetic findings have been associated with SRS. However, only chromosomes 7 and 17 are consistently involved in SRS. A subset of 7-10% of SRS patients shows complete or segmental maternal uniparental disomy of chromosome 7 (UPD(7)mat); additionally, five SRS patients have been described carrying rearrangements in 7p. Thus a central role of chromosome 7 in the aetiology of SRS can be delineated, while the contribution of chromosome 17 is uncertain. Disease causing mutations in genes on chromosomes 7 and 17 have not yet been identified. Nevertheless, the following diagnostic procedure should be offered routinely to elucidate causative genetic factors in SRS: after exclusion of chromosomal aberrations patients with the typical clinical signs of SRS (severe IUGR/PGR <P3; triangular face, clinodactyly V, relative macrocephaly, asymmetry) should be tested for UPD(7)mat using microsatellite markers localised in the candidate regions 7p12-p14 and 7q31-qter. To exclude cryptic deletions or duplications in 7p12-p14 escaping the aforementioned techniques we now established a real-time PCR approach based on TaqMan technology. We thereby quantified the copy number of GRB10 in 50 SRS patients, a gene in 7p which has almost been affected by the chromosomal disturbances reported for this region. By testing DNA of a patient with a duplication in 7p we could demonstrate the reliability and robustness of this test.

**Familial blepharophimosis and female sterility report in three generations in an Iranian family, Mashhad, North East of Iran.** *R. Akbarzadeh<sup>1</sup>, K. Ghodsi<sup>1</sup>, D. Farrukh Tehrani<sup>2</sup>, N. Khadem<sup>3</sup>.* 1) Department of Medical Genetics, Ghaem Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Khorasan, Iran; 2) Department of Ophthalmology, Ghaem Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Khorasan, Iran; 3) Department of Gynecology, Imam Reza Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Khorasan, Iran.

**Introduction:** Blepharophimosis/ptosis/epicanthus inversus syndrome (BPES) is a rare genetic disorder, but a well documented genetic condition that occurs sporadically and is transmitted by autosomal dominant inheritance that is characterized by distinctive eyelid abnormalities. This syndrome has been mapped to 3q23 chromosome. **Materials and Method:** To describe the clinical features, mode of inheritance, and linkage analysis twelve affected members of a three-generation family with BPES. Diagnosis was definite based on the clinical findings. Peripheral blood samples were taken and cells were harvested. GTG banding chromosomes were analyzed. **Results:** We reported on twenty-two individuals a family referred for genetic evaluation because of (BPES). Twelve of these patients had the blepharophimosis syndrome. Pedigree analysis showed autosomal dominant inheritance in this family. Ocular examination revealed residual ptosis, blepharophimosis and epicanthus inversus in both eyes. There was no history of consanguinity. The mother had not received any medication and had not been exposed to radiation during pregnancy. None of the members of this family showed evidence of other systemic disorders and no other abnormality was detected. Chromosomal analysis of peripheral blood was performed after high resolution banding showed an apparently normal. **Conclusion:** All currently reported cases of BPES show interstitial deletions or balanced translocations involving 3q22-q23. Our patients demonstrated an autosomal dominant, characteristic with a very high penetrance and expressivity, preferentially affecting and being transmitted by males. Affected females were infertile. It is possible that our patient has a contiguous gene defect including at least one locus for a type of blepharophimosis.

Program Nr: 740 from 2003 ASHG Annual Meeting

**Anophthalmia, Esophageal atresia and Genital anomalies: Three new cases.** *A.S. Schneider, T.M. Bardakjian.* Dept Genetics, Albert Einstein Medical Ctr, Philadelphia, PA.

The A/M Registry at Albert Einstein Medical Center (AEMC) was established in 1994 and has been offering eye development gene screening to registry participants since 1999. Testing involves mutational screening of 17 eye development genes in 8 participating labs in the US and Europe. To date we have collected clinical information on 200 cases in the Registry and DNA on 280 affected individuals. A review of the Registry revealed three patients with bilateral anophthalmia, esophageal atresia and genital anomalies. We will introduce three new cases of Anophthalmia and Esophageal Atresia Association. Since 1988, when Rogers first described a boy with esophageal atresia and anophthalmia, 9 patients with the association have been reported in the literature. Our cases appear to be distinct from those already described in the literature, thus increasing the number of cases with this association to twelve. Patient 1 is a 4-year-old male with bilateral anophthalmia, TE fistula and hypospadias who had a VSD repair. Case 2 is a 9-year-old male with bilateral anophthalmia, esophageal atresia and cryptorchidism. His clinical findings include hydrocephalus and holoprosencephaly. Case 3 is an 11-year-old male with bilateral anophthalmia, TE fistula, cryptorchidism and developmental delay. Two of these 3 patients have participated in the gene screening and no mutations have been identified to date. Further screening of these individuals is underway and we are in the process of obtaining DNA on Case 3 for analysis. We will describe the clinical findings in these patients and the gene studies performed including the identification of unusual polymorphisms in some cases. Our goal is to ultimately use these phenotypic descriptions to help guide the labs to screen specific genes.

**Familial Horizontal Gaze Palsy: Report of a UAE Family Demonstrating Autosomal Recessive Inheritance.** *E.I. Traboulsi<sup>1</sup>, L.J. Wang<sup>1</sup>, Q. Wang<sup>1</sup>, A. Mousawi<sup>2</sup>, E. Engle<sup>3</sup>.* 1) Cole Eye Institute, Cleveland Clinic Foundation, Cleveland, OH; 2) Al-Jazeirah Hospital, Abu Dhabi, United Arab Emirates; 3) Departments of Genetics and Neurology, Boston Children's Hospital, Harvard Medical School.

**Purpose:**To report a UAE family with 5 siblings affected with horizontal gaze palsy. **Methods:** Clinical examination and surgery on four affected members of a family comprised of five affected siblings, five unaffected siblings and consanguineous parents. **Results:** Two brothers and two sisters (ages 4 - 17 years) had congenital bilateral esotropia and abnormal eye movements since birth. The clinical manifestations were identical in all four patients. There was no ptosis. Abduction was severely limited bilaterally. Vertical ocular movements were normal. There were convergence-like eye movements on attempted abduction in lateral gaze. Visual acuity was very good in all patients, reflecting probable alternating fixation. Forced duction testing under general anesthesia revealed very tight medial rectus muscles in all patients. None of the children had severe scoliosis. Bilateral medial rectus recession resulted in good ocular alignment in primary position of gaze without any alteration of the abnormal ocular movement pattern. **Conclusions:** This large family appears to have the rare disorder known as recessive horizontal gaze palsy with progressive scoliosis.

**Atypical X-Inactivation in an X;1 Translocation Patient with Oto-Palato-Digital Syndrome.** *C.E. Cottrell<sup>1,2</sup>, A. Sommer<sup>1,2</sup>, G.D. Wenger<sup>1,2</sup>, K. Krahn<sup>3</sup>, A. Modesto<sup>3</sup>, L. Van Westen<sup>3</sup>, S. Bullard<sup>3</sup>, W.R. Wilcox<sup>4</sup>, T. Ogata<sup>5</sup>, A.C. Lidral<sup>3</sup>, J.M. Gastier<sup>1,2</sup>.* 1) Columbus Children's Hospital, Columbus, OH; 2) The Ohio State University, Columbus, OH; 3) Univ. of Iowa, Iowa City, IA; 4) Cedars-Sinai/UCLA, Los Angeles, CA; 5) Natl Inst for Child Health and Dev., Tokyo, Japan.

X-inactivation is a critical mechanism which allows for dosage compensation in humans. Transcriptional silencing of one X-chromosome occurs in female somatic cells to prevent overexpression of X-linked genes. X-inactivation is random, but in cases of an X;autosome translocation, the normal X is inactivated to prevent monosomy of autosomal genes. Oto-palato-digital (OPD) syndrome is an X-linked (Xq28) craniofacial condition involving cleft palate, hearing loss, and multiple skeletal anomalies. Recently, Robertson et al. reported that mutations in the FLNA gene (Xq28) cause OPD [Nature Genet. 33, 487-491 (2003)]. The mutations are described as having gain-of-function effects on the protein product, filamin A. A patient with a translocation [46,X,t(X;1)(q28;q21)de novo] and clinical features of OPD was studied to characterize the molecular cause of OPD. **Purpose:** To characterize the etiology of OPD syndrome in the patient. **Methods:** The research plan included FISH to narrow the critical region, PCR analysis of somatic cell hybrids, RT-PCR using candidate genes, and X-inactivation studies of the patient. **Results:** The patient was found to have a breakpoint in the DKC1 gene (400kb downstream of FLNA). The patient has an extremely skewed pattern of X-inactivation, with preferential inactivation of the derivative X. RT-PCR results showed that both DKC and FLNA are being expressed. A PCR analysis of FLNA ruled out large deletions in the patient. **Conclusions:** Despite being clinically diagnosed with OPD syndrome, the etiology of the disease remains unclear in this patient. Functional disomy of genes in the Xq28 region, in addition to partial monosomy of chromosome 1q are possible consequences of the patients X-inactivation pattern. If the mutations in FLNA which cause OPD are truly gain-of-function (as reported in Robertson, et al.), it is possible that our patients phenotype is caused by overexpression of filamin A protein.

**Gonadal Mosaicism in Incontinentia Pigmenti: a case report.** *K.L. Chong<sup>1</sup>, B.R. Krafchik<sup>2</sup>, B.B. Roa<sup>3</sup>*. 1) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hosp, Toronto, ON, Canada; 2) Hospital for Sick Children Toronto, ON, Canada; 3) Department of Human and Molecular Genetics, Baylor College of Medicine, Houston, Texas.

Incontinentia pigmenti (IP) is an X-linked dominant condition characterized by changes to the skin, hair, teeth and nails. Cognitive delays are occasionally seen. IP is lethal in most, but not all males. Gonadal mosaicism has been previously demonstrated in a father. We report a couple with two daughters with classical dermatological findings of IP. The older daughter is 5 years old and has skin findings, conical teeth, thin hair and pitted nails. Her development is normal to date. Molecular testing for the common deletion in the IKBKG gene is found in approximately 80% of female patients affected with IP. This deletion was absent in our patient as well as in her parents suggesting a de novo mutation in the IP gene. The couple's second daughter was born in 2002 and had skin findings at birth strongly suggestive of IP. This mother is not affected clinically with IP and both parents have negative molecular testing. Thus, the possibility of gonadal mosaicism in either parent is hypothesized.

**A new family with X-Linked Lissencephaly: description of Brazilian family.** *C.D. Martinhago<sup>1</sup>, A.C. Laus<sup>2</sup>, G.N. Simao<sup>3</sup>, L. Martelli<sup>1,2</sup>, J.M. Pina Neto<sup>1,2</sup>.* 1) Medical Genetics, HCFMRP - University of Sao Paulo, Brazil; 2) Dept. Genetics, School of Medicine of Ribeirao Preto - University of Sao Paulo, Brazil; 3) Dept. of Radiology, HCFMRP - University of Sao Paulo, Brazil.

X-linked lissencephaly and subcortical band heterotopia is familial neuronal migration disorder secondary with DCX mutations. The gene, doublecortin (DCX), is located in Xq22.3. Males have very limited development with neurological findings that begin soon after birth, develop seizures of variety types, hypotonia and spasticity. Female, however, often have mild to moderate mental retardation, but some have normal intelligence. Besides that, females usually have mild alterations with subcortical laminar heterotopia and severe form in males with agyria-pachygyria complex, like in this family, where a female has mild alterations in cerebral cortex than male. The LIS1 gene, located in 17p13.3, can be also associated with classical lissencephaly. In X-linked LIS, the malformation is more severe in the frontal lobes, whereas in chromosome 17, LIS severity is maximal in the occipital lobes. We reported a family, mother (patient 1), her two sons (patient 3 and 4) and their cousin (patient 6), that show the X-linked LIS abnormalities. The brain MRI study showed remarkable similarity structural alterations in the frontal lobes, characterized by abnormal thick cortex with broad but shallow gyri and a poorly defined boundary of the cortex and white matter, defined as pachygyria. These abnormalities were observed in patients 3, 4, 1 and 6 (two brothers, mother and a cousin). Patient 1 presents a mild form of pachygyria. All patients with this abnormal cortex present a subcortical band of gray matter, a laminar heterotopia. Patients 4, 1 and 6, the laminar heterotopia, was localized in frontal anterior lobes and in patient 3 in parietal lobes. The cortical sulci overlying the bands often appear abnormally shallow. There were areas with malformations between band heterotopia and pachygyria. Cytogenetic and FISH (Miller-Dieker 17p13.3 probe) studies were performed on peripheral lymphocytes of patient 1,3 and 4 which results were normal. Supported by: FAEPA-HCFMRP, University of Sao Paulo.

**FMRP, *FMRI*-mRNA, and psychopathology correlations in women with the fragile X premutation.** *S.W. Harris, F. Tassone, I. Barbato, R.J. Hagerman.* M.I.N.D. Institute, UC Davis Medical Center, Sacramento, CA.

Females with the premutation typically have a normal IQ, although approximately 25% may have limited physical features of fragile X, such as prominent ears, or psychological problems, such as depression or anxiety. We have only rarely seen the fragile X tremor/ataxia syndrome (FXTAS) in females. The presence of FXTAS in males is thought to be related to a gain of function toxic effect of elevated mRNA levels. Since it has been previously reported that females with the premutation also have elevated *FMRI*-mRNA levels, we wanted to explore whether the psychological features in premutation carrier females are related to molecular parameters, including *FMRI*-mRNA and FMRP levels.

We studied 10 females with the *FMR1* premutation, and carried out molecular analysis, including immunocytochemical FMRP levels and *FMR1*-mRNA levels. We also administered several cognitive and psychological measures, including the Symptom Checklist-90-Revised (SCL-90-R), which is a measure that quantifies several domains of psychopathological functioning. We found that FMRP was negatively correlated ( $p=.04$ ) with the depression domain on the SCL-90-R, such that lower levels of FMRP predicted higher scores, or more psychopathology, on the depression scale of the SCL-90-R. There was no significant relationship found between *FMR1*-mRNA level and any of the domains of the SCL-90-R. Thus far, these data suggest that depression is related to FMRP deficits and not to a toxic effect of elevated mRNA, and we plan to expand our analysis to over 30 subjects, so that we will gain a broader picture of the clinical-molecular phenotype in these women.

**A severe form of X-linked encephalopathy maps to chromosome Xp22.** *G. Viot*<sup>1</sup>, *I. Giurgea*<sup>2</sup>, *I. Desguerre*<sup>3</sup>, *A. Munnich*<sup>2</sup>, *P. de Lonlay*<sup>2</sup>. 1) Dept Genetics, Hopital Necker-Enfants Malades, Paris, France; 2) INSERM U393, Hopital Necker-Enfants Malades, Paris, France; 3) Dept Pediatrics, Hopital Necker-Enfants Malades, Paris, France.

We report a novel form of X-linked encephalopathy in a family of seven affected males over five generations. The affected males had cholestasis at birth, followed by hepatomegaly with steatosis and coarse facies. The neurological development was normal until one month, then the affected boys developed myoclonic seizures, dystonia, abnormal eye movement and dead in the first year of life. MRI investigation displayed white matter and brain stem abnormalities. Plasma (uricemia, very-long-chain fatty acid, phytanic and pipecolic acid) and urinary investigations (amino acids and organic acids, sulfites, oligosaccharides, mucopolysaccharides) were normal. Lactate, neurotransmitters, amino acid, glucose, creatine and guanidino-acetate in the CSF were normal as well. Visual evoked potentials, electroretinogram and respiratory chain enzyme activities in skeletal muscle and liver biopsies were unremarkable. Liver electronic microscopy revealed abnormal peroxysomes with vacuole density abnormalities. A chromosome X-wide linkage analysis allowed us to map the disease gene to chromosome Xp22 between DXS8051 and DXS8027 (Z=2, =0). This 15 Mb region encompasses the Serine/Threonine Kinase gene (STK9) which accounts for a mental retardation and West syndrome. The sequencing of this gene is in process. To date, not similar clinical presentation with severe encephalopathy and early death has been mapped to this region.

**Subtelomeric rearrangements in cases with mental retardation and dysmorphic features.** *C. Lozzio, T. Ryan, E. Bamberger, W. Carter, E. Ralston, C. Worthington, M. Huckaba.* Dept Medical Genetics, Univ Tennessee, Knoxville, TN.

Subtelomeric aberrations were studied using Vysis ToTelVysion probe panel. Abnormalities were detected in cases with normal karyotypes and the chromosomes involved in "de novo" rearrangements were identified. A 2 years old female with deletion 4p had a milder phenotype than the classical Wolf-Hirschhorn syndrome and showed developmental delay, failure to thrive, dysmorphic features and microcephaly. A deletion 4q was found in a 4 years old female with speech delay and dysmorphic features. A deletion 9q was observed in a 9 years old female with moderate mental retardation, autistic behavior, dysmorphic features and microbrachycephaly. A deletion Yq12 and trisomy Yp11.3 were observed in a 4 years old child with developmental delay and radioulnar synostosis. The father had a normal Y. A deletion 22q13.3 was found in a 4 years old female with developmental delay, ataxic gait, obesity and dysmorphic features. A der(13)t(5;13)(p15.3;q34)(tel5pX3,tel13qX1) was seen in a 4 years old female with developmental delay, microcephaly and Factor VII deficiency. Parental studies were normal. A der(1)t(1;8)(p36.3;q24.3)(1ptel-;8qtel+) was found in a 4 years old male with severe IUGR, microcephaly, developmental delay, ataxic gait and multiple dysmorphic features. A half cryptic "de novo" translocation with der(8)t(8;13)(p23;q22) was found in a 14 months old male with craniofacial asymmetry, developmental delay and hypotonia. Translocations of the telomeric regions 7q and 11p were observed in cases with apparently balanced karyotypes and abnormal phenotypes: two translocations of the 7q region included a complex rearrangement of chromosomes 2 and 7 in a 15 months old girl with infantile spasms, developmental delay, hypotonia and a t(7;8)(q36.3;q22.3) in a child with cleft lip and palate, hypodontia, microcephaly, and seizures. An inherited t(4;11)(q23;p15.30) was seen in the normal mother and in her child with Beckwith Wiederman syndrome (BWS). Maternal imprinting of the BWS region may explain these findings. These cases are examples of the types of patients in which studies with subtelomeric probes can detect or help to interpret chromosome aberrations of clinical significance.

**Cytogenetic Approaches to Finding Auditory Genes.** *R.E. Williamson<sup>1</sup>, W. Lu<sup>1,2</sup>, S. Michaud<sup>1,2</sup>, R.E. Peters<sup>2</sup>, B.J. Quade<sup>1,2</sup>, A.M. Michelson<sup>1,2,3</sup>, R.L. Maas<sup>1,2</sup>, C.C. Morton<sup>1,2</sup>.* 1) Harvard Medical School, Boston, MA; 2) Brigham and Women's Hospital, Boston, MA; 3) Howard Hughes Medical Institute.

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 performed 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)]. Breakpoints were assigned initially by GTG-banding and BAC probes for FISH experiments to map precisely the breakpoints were chosen based on proximity to the assigned 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 in FISH experiments and in Southern blot analysis. The chromosome 8 breakpoint disrupts a hypothetical annotated sequence and, on chromosome 9, a known gene, methylthioadenosine phosphorylase (*MTAP*), is located in the breakpoint region. It is hypothesized that a disruption of *MTAP* would lead to deafness due to its role in metabolizing an inhibitor of polyamine synthesis. The other case, DGAP056, has a translocation between chromosome 2 and chromosome 13 [t(2;13)(p24;q21)]. Initial analyses suggest that the chromosome 2 break is within the sequence for a hypothetical gene, *FLJ21820*. This gene is expressed in the cochlea and Northern blot analysis shows that it is more highly expressed in fetal tissues than in adult tissues. A knock out mouse is being created to establish the role of *FLJ21820* in the auditory system.

**Y chromosome microdeletions in male infertility.** *N.B. Abdelmoula<sup>1</sup>, L.A. Keskes<sup>1</sup>, T. Bienvenu<sup>2</sup>, T. Rebai<sup>1</sup>, A. Bahloul<sup>3</sup>, H. Ayadi<sup>4</sup>.* 1) laboratoire d'histologie, facult de mdecine de sfax, sfax, Tunisia; 2) Laboratoire de biochimie et de genetique moleculaire, Hpital Cochin AP-HP Paris, France; 3) Service d'urologie, CHU H. Bourghuiba Sfax, Tunisia; 4) Laboratoire de genetique moleculaire humaine facult de mdecine de sfax, sfax, Tunisia.

Microdeletions in Yq11 overlapping azoospermia factors (named AZFa, AZFb, AZFc and AZFd for azoospermia factors a, b, c and d) are recurrently detected in about 10-15% of idiopathic azoospermia and severe oligozoospermia. AZFc deletions, involving the DAZ gene, form the majority of these deletions. Screening for Y chromosome microdeletions is often performed by analyzing the presence of Y chromosome-specific markers using multiplex PCR. This strategy requires many precautions to minimize the number of false-negative or false-positive results. So, it becomes limiting in a clinical context. In this study we used a rapid molecular genetic strategy to identify DAZ gene deletion in a group of 36 infertile patients. The experiment consists of amplifying simultaneously DAZ and DAZ like-autosomal genes followed by a separation of the two PCR products with good resolution using denaturing gradient gel electrophoresis (DGGE). DAZ microdeletions were detected in one patient among 20 oligospermic men (5%) and in two patients among 16 azoospermic men (12,5%). Our finding suggest that Y microdeletions, especially at AZFc region, occur frequently in idiopathic male infertility and demonstrate that PCR-DGGE method for detection of DAZ gene deletion could be particularly useful for screening of the DAZ locus in the diagnosis workup of nonobstructive azoospermia and severe oligozoospermia in infertility clinics. Furthermore, they support the recommendation to perform genetic defect screening among infertile men before their enrolment an ICSI/IVF program.

**Sex-chromosome mosaicism in azoospermic men.** *A. Amouri<sup>1</sup>, N.B. Abdelmoula<sup>2</sup>, M.F. Portnoi<sup>3</sup>, A. Bahloul<sup>4</sup>, L. Keskes<sup>1</sup>, A. Saad<sup>5</sup>, T. Rebai<sup>1</sup>.* 1) LABORATOIRE DE CYTOGENETIQUE, INSTITUT PASTEUR DE TUNIS Tunisia; 2) LABORATOIRE D'HISTOLOGIE, FACULTE DE MEDECINE DE SFAX Tunisia; 3) LABORATOIRE DE CYTOGENETIQUE, HOPITAL SAINT-ANTOINE PARIS France; 4) SERVICE DUROLOGIE, HOPITAL HABIB BOURGUIBA SFAX Tunisia; 5) LABORATOIRE DE CYTOGENETIQUE HOPITAL FARHAT HACHED SOUSSE Tunisia.

Patients with azoospermia have an elevated impact of numerical abnormalities of X chromosome. mosaic have less severe features and may be fertile, as there may be a normal clone of the cells within the testes. They can be proposed for ICSI with testicular sperm extraction (TSE) after genetic counselling. Nonmosaic patients can also (but less better than mosaic patients) rely in ICSI with TSE and then become fathers. The aim of this study was to detect sex chromosome abnormalities among azoospermic men and to evaluate the sensitivity of fluorescence in situ hybridization (FISH) techniques to assess mosaicism mainly in Klinefelters patients in comparison with classical cytogenetic methods. Among 21 azoospermic patients who were karyotyped, 10 showed a numerical sex chromosome abnormalities. Three of them have mosaic karyotype with two cells populations: 46,XY and 47,XXY. The others were homogeneous: 46, XX (one patient) and 47,XXY (six patients). Using fluorescence in situ hybridization techniques for all klinefelters patients only one among 3 showed evidence of sex chromosomal abnormalities mosaicism. We suggest that FISH analysis should be applied when sex chromosome mosaicism is shown by G or R-banding technique. We suggest that FISH analysis which is a powerful tool for detecting more sensitively mosaicism in klinefelters syndrome is recommended as a complementary test before discussing prognosis and expected risk of chromosomal aberrations in the offspring after ICSI.

**Sequential cytogenetic and molecular cytogenetic characterization of an *SV40T* immortalized nasopharyngeal cell line transformed by Epstein-Barr virus latent membrane protein-1 gene.** C. Jin<sup>1</sup>, H. Zhang<sup>2, 5</sup>, S.W. Tsao<sup>4</sup>, B. Strömbeck<sup>1</sup>, P.W. Yuen<sup>2</sup>, Y. Kwong<sup>3</sup>, Y. Jin<sup>1, 2, 3</sup>. 1) Department of Clinical Genetics, University Hospital, Lund, Sweden; 2) Departments of Surgery, Queen Mary Hospital, University of Hong Kong, Medical Centre, Hong Kong, China; 3) Departments of Medicine, Queen Mary Hospital, University of Hong Kong, Medical Centre, Hong Kong, China; 4) Department of Anatomy, Faculty of Medicine, University of Hong Kong, Hong Kong, China; 5) Department of Otolaryngology, Ruijin Hospital, Shanghai Second Medical University, Shanghai, China.

Cytogenetic and molecular cytogenetic analyses were performed on four sublines derived from a newly established, *SV40T* immortalized nasopharyngeal (NP) cell line, NP69, with two of the sublines expressing *LMPI*, an EBV encoded gene. A total of 7 cytogenetically related subclones were identified, all having highly complex karyotypes with massive numerical as well as structural rearrangements. Centromeric rearrangements, in the form of isochromosomes and whole-arm translocations, were prevalent. Cytogenetic sign of gene amplification, i.e., homogeneously staining region (HSR), was detected at chromosomal loci 1q21 in all metaphase cells analyzed. Multicolor COBRA-FISH was used to confirm the karyotypic interpretations. Furthermore, multicolor COBRA-FISH showed also that part of the HSR contained chromosome 20 material. Extensive clonal evolution could be observed by the assessment of karyotypic variation among different subclones and individual metaphase cells. The evaluation of clonal evolution enabled the temporal order of chromosome aberrations during cell immortalization and malignant transformation to be identified. A striking karyotypic similarity was found between sublines expressing *LMPI* and an NPC cell line, with loss of genetic material from chromosome arm 3p being an important recurrent observation. More interestingly, the karyotypic features of NP69 were also similar to those of many epithelial malignancies. Our observations suggest that serial transformation of NP cell lines might provide a useful in vitro model for the study of the multistep neoplastic transformation of NP cells.

**A case of infantile ALL presenting with rearrangement of MLL at 11q23 and apparent insertion/translocation at 10p(12).** *C. Tirado<sup>1</sup>, J. Lager<sup>2</sup>, V. Golembiewski-Ruiz<sup>1</sup>, P. Rosoff<sup>2</sup>, J. Gong<sup>1</sup>, B. Goodman<sup>1</sup>.* 1) Pathology. Duke Univ Med Ctr, Durham, NC 27710; 2) Division of Hematology-Oncology-Department of Pediatrics. DUMC-Durham, NC 27710.

We report an 11 month old patient with infant ALL who presented circulating lymphoblasts, history of diarrhea, paleness and poor oral intake. Her white blood cell count was  $18 \times 10^3/l$  and hemoglobin 5.5g/dL. Bone marrow analysis showed that 80% of the nucleated cells were lymphoblasts. Four drug induction therapy was initiated (Berlin-Frankfurt Munster protocol). FISH studies using the MLL break apart probe from Vysis (Downers Grove, IL) showed rearrangement within the MLL locus (11q23) in 69% of the cells examined. A very subtle rearrangement involving the short arm of chromosome 10 and the long arm of chromosome 11, was seen by routine chromosome analysis and it could only be detected with certainty in 14% of the cells examined. To clarify the nature of the MLL rearrangement, the break apart probe was also applied to previously G-banded slides. In light of the FISH studies, this rearrangement was interpreted as a small inversion within 11q23 separating the 5'MLL from the 3'MLL region. The segment on 11q containing the rearranged MLL locus was then inserted/translocated to the short arm of chromosome 10 at approximately band 10p12. There have been previous reports of rearrangements of MLL with the MLLT10 gene locus at 10p12, including interstitial inverted insertion of 11q13q23, in one case or 11q14q23 in another case at 10p12. These both resulted in a derivative chromosome 10 and transcription of an MLL/MLLT10 fusion product. Because of the limited material available in our case, further FISH and molecular characterization were not possible. To our knowledge, the novel, very cryptic rearrangement detected in our patient has not been described previously. A follow up study of the bone marrow at the end of induction revealed no morphological evidence of residual leukemia and both FISH and chromosome analyses were normal. This case illustrates the importance of performing MLL FISH in addition to routine cytogenetic analysis when the differential diagnosis includes an MLL positive leukemia.

**DiGeorge syndrome in a patient with isochromosome 18p.** *R. Deberardinis, L. Medne, E.H. Zackai.* Div Human Gen & Molec Biol, The Children's Hospital of Philadelphia, Philadelphia, PA.

DiGeorge syndrome (DGS) is a well-known phenotype in which conotruncal heart defects, hypocalcemia, thymic hypoplasia, and characteristic dysmorphisms are major clinical features. The phenotype results from abnormal development of the third and fourth branchial arches. The 22q11.2 deletion syndrome is the best-characterized etiology of DGS, although other chromosomal abnormalities, including deletions affecting chromosome 10p13, have been reported in a few patients. We present a patient with interrupted aortic arch type B (IAA-B), neonatal hypocalcemia, thymic hypoplasia, and dysmorphic features including microcephaly, thick, overfolded helices, long fingers and toes, and anteriorly-placed anus. Cytogenetic studies revealed presence of a marker chromosome, identified by FISH as an isochromosome 18p. We did not detect a 22q11.2 deletion by FISH using a cosmid probe corresponding to locus D22S75. In retrospect, the patients dysmorphisms were consistent with the tetrasomy 18p syndrome, but this is the first case suggesting that tetrasomy 18p is a DGS phenocopy. The case is particularly interesting with respect to the patients heart defect. While 50% of patients with IAA-B have a 22q11.2 deletion, this rare conotruncal anomaly is not clearly associated with any other cytogenetic abnormality and has not been seen in any patients with tetrasomy 18p.

**Genomic alterations in the endometrium may be a proximate cause for endometriosis.** *S.W. Guo<sup>1</sup>, Y. Wu<sup>1</sup>, E. Strawn<sup>1</sup>, Z. Basir<sup>1</sup>, Y. Wang<sup>2</sup>, G. Halverson<sup>1</sup>, K. Montgomery<sup>1</sup>, A. Kajadacsy-Balla<sup>1</sup>.* 1) Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI; 2) University of California, Santa Barbara, CA.

One popular theory on the pathogenesis of endometriosis is Sampson's theory that retrograde menstruation causes endometriosis. Since retrograde menstruation is nearly universal, however, there is an apparent paradox as why only a fraction of women develop endometriosis. We hypothesize that certain genomic alterations in the endometrium may predispose women to endometriosis. This hypothesis can well explain the paradox, and may also explain why some women develop microscopic lesions while others have more serious endometriosis. To test this hypothesis, we carried out a genomic analysis of endometrial tissue samples from women with endometriosis. Endometrial tissue samples were taken from five women with endometriosis. For controls, we used endometrial tissue samples from four women who underwent elective abortions and one sample from placenta. Using array-based comparative genomic hybridization (CGH), we determined the normal range of variation in CGH signals using normal controls. CGH results were further confirmed by real-time quantitative PCR and loss of heterozygosity (LOH) analysis. We identified several regions of genomic alterations in all five patients. Some of these regions were the same regions identified previously in endometriotic lesions. For select markers, the genomic alterations were confirmed by real-time PCR and LOH analyses. Therefore, there is evidence that the endometrium in women with endometriosis has genomic alterations and that genomic alterations found in endometriotic lesions may originate from endometrium. This is consistent with numerous reports that the endometrium of women with endometriosis differ from those of women without. Our finding suggests that genomic alterations in the endometrium may be a proximate cause for endometriosis.

### **Characterization of the Chromosomal Duplication Associated with Hereditary Benign Intraepithelial**

**Dyskeratosis.** *W. Lambert<sup>1</sup>, M. Maready<sup>1</sup>, M. Bembe<sup>1</sup>, H. Xu<sup>1</sup>, R. Allingham<sup>2</sup>, J. Vance<sup>1</sup>, M. Hauser<sup>1,2</sup>.* 1) Center for Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Department of Ophthalmology, Duke Univ Medical Ctr, Durham, NC.

**Purpose:** Hereditary benign intraepithelial dyskeratosis (HBID) is an autosomal dominant cell proliferation disorder characterized by opaque epithelial plaques of the conjunctiva and oral mucosa. Found primarily among a Native American tribe in North Carolina and their relatives, HBID plaques appear at birth or in early childhood. Involvement typically includes the conjunctiva, cornea, and buccal mucosa. Loss of vision secondary to corneal involvement can occur. Recently, this disorder was linked to chromosome 4q35 and the presence of three microsatellite alleles in affected individuals revealed a near-telomeric DNA duplication in this region (Allingham RR, et al. *Am J Hum Genet* 2001).

The proximal duplication endpoint was found to lie between markers D4S2299 and D4S2390 (227 kb). The purpose of this study was to further localize the proximal duplication endpoint. **Methods:**

Tandyman([www.stdgen.lanl.gov/tandyman.index.html](http://www.stdgen.lanl.gov/tandyman.index.html)) was used to identify seven novel, potentially polymorphic microsatellite markers between D4S2299 and D4S2390. Analysis of a large HBID family (27 individuals, 16 affected, 7 non-affected, and 4 potentially affected) and a control population (48 individuals) was performed using these new markers.

**Results:** Two markers located 30 kb and 64 kb proximal to D4S2390 resulted in duplicated alleles in all HBID affected individuals and in two potentially affected individuals. No duplicated alleles were observed in the control population for any of the seven markers analyzed. **Conclusions:** Based on these results the proximal duplication endpoint is located within a 46 kb region on 4q. Southern blotting will be performed to further localize the duplication endpoint. A lambda phage-based genomic library is being constructed from patient DNA in order to clone and sequence the duplication endpoint.

**Mitotic Index and Dementia Status in Down Syndrome.** *E.C. Jenkins, L. Ye, S. Li, W.B. Zigman, N. Schupf, E.A. Sersen, N. Zhong, C.M. Miezieski, S. Sklower Brooks, W.P. Silverman.* NYS Inst Basic Res Dev Disab, Staten Island, NY.

Previously, we reported a decrease in mitotic index (MI) of skin fibroblast cultures from five people with Alzheimer's Disease (DS) (Jenkins et al., 1998). Now, we have found decreased MIs in short-term whole blood cultures from 12 of 15 people with trisomy 21 and dementia compared to 13 individuals with trisomy 21 who were not demented ( $p < .01$ ). The non-demented individuals were generally age- and sex-matched with demented individuals with DS. An MI  $\leq 25$  suggested dementia while an MI  $> 25$  suggested non-dementia. Only one of the 13 non-demented trisomy 21 individuals exhibited an MI of  $\leq 25$ . The individuals with and without dementia ranged in age from 50-66 years with a mean ages of 56.95, and 55.95 for dementia and non-dementia, respectively. All classifications of dementia were consistent with the recommended criteria of the American Association on Mental Retardation, the International Association for the Scientific Study of Intellectual Disability, and the World Health Organization. Additional preliminary studies with a second group of non-demented individuals with DS but who also exhibited "mild declines" also suggested that an MI  $\leq 25$  would indicate mild decline versus generally age- and sex-matched individuals with no dementia or decline and DS ( $p = .04$ , two-tailed). This group of individuals ranged in age from 50 to 73 years with a mean age for females with cognitive decline of 54.60 years while the age for females with no decline was 53.4. Our preliminary findings indicate that reduced MI may be associated with dementia and cognitive decline in adults with DS. We will now evaluate additional individuals with dementia or with preliminary signs that may lead to dementia, such as mild declines, in order to confirm our observations reported here and to determine whether MI may be used as a marker for the potential development of dementia. (This work was supported in part by the NYS Office of Mental Retardation and Developmental Disabilities, Alzheimer's Association Grant IIRG-09-1598, and by NIH grants PO1 HD35897, HD37425, and RO1 AG014673.).

**DNA studies of mono-and pseudodicentric isochromosomes 18q.** *M. Bugge*<sup>1, 2</sup>, *C.A. Brandt*<sup>3</sup>, *M.B. Petersen*<sup>2, 4</sup>. 1) Wilhelm Johannsen Centre for Functional Genome Research, Dept Medical Genetics, Univ Copenhagen, Copenhagen, Denmark; 2) Department of Medical Genetics, The John F Kennedy Institute, Glostrup, Denmark; 3) Department of Clinical Genetics Vejle County Hospital, Denmark; 4) Department of Genetics, Institute of Child Health, Athens, Greece.

The clinical syndrome associated with isochromosome 18 (both mono-and pseudodicentric) is very similar to Edwards syndrome (trisomy 18). The parental origin has only been investigated in a very few cases so far and very little is known about the mechanisms of formation. We have diagnosed four cases with de novo isochromosomes 18q by conventional cytogenetics techniques in combination with molecular cytogenetics investigations. One case was a fetus diagnosed at prenatal diagnosis due to advanced maternal age, and three cases were liveborn children. Two cases were monocentric and two pseudodicentric. We used 23 microsatellite DNA polymorphisms covering the entire length of chromosome 18 to determine the parental origin and mechanisms of formation. In one of the monocentric cases the isochromosome had two identical q arms of maternal origin. The other monocentric case had 48 chromosomes with supernumerary isochromosomes i(18p) and i(18q) of maternal origin, probably formed by postzygotic nondisjunction of chromosome 18 followed by centromeric misdivision. In the two dicentric cases the isochromosomes had genetically identical arms composed of a part of the short and the whole long arm of chromosome 18 and were of paternal origin. The formation of the dicentric isochromosomes can be explained by postzygotic exchange of sister chromatids on the short arm of chromosome 18 (breakage and U-shape reunion).

**A 13 Mb deletion on 2q21 in a patient with a t(1;2)(p31;q21), autism and multiple congenital anomalies.** *L. Edelmann*<sup>1</sup>, *A.L. Shanske*<sup>2</sup>, *P. Gosser*<sup>3</sup>, *N.B. Kardon*<sup>1</sup>, *B. Levy*<sup>1</sup>. 1) Dept Human Gen, Box 1497, Mt Sinai Sch Medicine, New York, NY; 2) Children's Hospital at Montefiore, Albert Einstein College of Medicine, Bronx, NY; 3) Dept de Genetique, et Unite INSERM U-393, Hopital Necker-Enfants Malades, Paris, France.

Comparative Genomic Hybridization (CGH) was previously used to detect the loss of chromosomal material from chromosome 2 in a child with a denovo translocation t(1;2)(p31;q22). The child presented with multiple congenital anomalies, chronic intestinal obstruction and mental retardation with severe language delay that was later diagnosed as autism. Reports of chromosome 2 deletions are rare in the literature; the most notable is a case of Mowat-Wilson syndrome (MWS) with a del(2)(q21q23). Mutations in the ZHFX1B gene, located on 2q22, were recently shown to cause MWS. To determine whether the deletion in this translocation patient included ZHFX1B, Fluorescence in Situ Hybridization (FISH) analysis was performed using a BAC clone containing this gene, RP11 9509. This BAC was not deleted in the patient and was present on the der(1) chromosome, indicating that the deletion was proximal to this region. To confirm the original CGH results and delineate the size and boundaries of the deletion, we performed FISH analysis using a total of nine BAC clone probes that span approximately 18 Mb from 2q14 to 2q22. The breakpoint on 2q14 was narrowed to the region flanked by BACs, RP11 270M20 (not deleted) and RP11 314L11 (deleted), which are 100 kb apart on genomic contig NT\_022135, at positions 124.4 Mb and 124.5 Mb, respectively. The breakpoint on 2q21 was narrowed to a region on BAC RP11 171B14 located at position 138.2 Mb, as hybridization to the der(2) chromosome was consistently weaker than to the normal 2. Therefore, the size of the deletion in this patient is approximately 13 Mb and spans almost the entire 2q21 band. This region of the genome contains hundreds of genes and the complex phenotype in this patient may result from a contiguous gene deletion, however, these results provide valuable information with respect to loss of 2q21 chromosomal material and may help to delineate a potential autism susceptibility region.

**De novo trisomy Xq28-qter detected by subtelomeric FISH screening.** *M.G. Bialer<sup>1</sup>, A. Anguiano<sup>3</sup>, I. Taff<sup>2</sup>, A. Shanmugham<sup>1</sup>, D. LaGrave<sup>3</sup>, B.J. White<sup>3</sup>.* 1) Div Med Genetics and; 2) Ped Neurology, North Shore Univ Hosp/NYU Med Ctr, Manhasset, NY; 3) Quest Diagnostics' Nichols Institute, San Juan Capistrano, CA.

An 18 mo old female was evaluated for microcephaly and developmental delay. Delivery was induced due to oligohydramnios. Birthweight was 1990 g at 36 wk gestation. The patient failed to thrive and developed hypotonia and microcephaly. At her 18 mo evaluation, length was 72.5 cm (5%), weight 9.24 kg (5-10%) and OFC 41.5 cm (5%). She had severe trigonocephaly, occipital flattening, epicanthal folds, wide nasal bridge, small downturned mouth, thickened maxillary alveolus and hypotonia. Developmentally, she babbled and razzed. She could roll and hold a sit. The child died at 24 mo of RSV pneumonia. High resolution blood chromosome analysis, metabolic workup, serum very long chain fatty acids, MECP2 gene sequencing and MRI scan of the brain were normal. Subtelomeric FISH screening revealed an unbalanced translocation of terminal Xq to 13p. FISH with subtelomeric probe (Tel Xq), whole X chromosome painting and centromeric probe identification were consistent with this result. Both parents had normal subtelomeric FISH studies. Usually an unbalanced translocation results in duplication of one chromosomal segment and deletion of another. It therefore can be difficult to determine which clinical features are caused by a specific chromosome anomaly. This child had trisomy of Xq28 without a clinically significant deletion, so her clinical features are entirely related to partial trisomy Xq28-qter. One of us (MGB) was co-author of a previous report of 4 boys with duplication of Xq27-qter onto distal Xp (Goodman BK et al, Am J Med Genet 1998;80:377-84). Like the subject of this report, their findings included prenatal onset growth retardation, microcephaly, developmental delay, hypotonia, epicanthal folds, relative hypertelorism and downturned mouth, but their growth problems were more severe. Two of them have died of pneumonia and a 3rd has frequent pneumonias. The present patient's result shows that their features are mainly related to trisomy Xq28 and adds a new finding, trigonocephaly, to the list of phenotypic features of this chromosome anomaly.

**Duplication 20q: Clinical, Cytogenetic and Array Comparative Genomic Hybridization Characterization of a New Case.** *P.D. Cotter*<sup>1,2</sup>, *A. Iglesias*<sup>3</sup>, *K.A. Rauen*<sup>2,4</sup>, *D.G. Albertson*<sup>4,5</sup>, *D. Pinkel*<sup>4,5</sup>. 1) Department of Pathology, Childrens Hospital Oakland, Oakland, CA; 2) Department of Pediatrics, University of California San Francisco, San Francisco, CA; 3) Nassau University Medical Center, East Meadow, NY; 4) Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA; 5) Department of Laboratory Medicine, University of California San Francisco, San Francisco, CA.

Trisomy of the long arm of chromosome 20 is rare, with most cases the result of malsegregation of a parental translocation resulting in trisomy for variable regions of 20q. There is only one previous report of an interstitial duplication of the proximal long arm of chromosome 20. However no clinical information was provided. Consequently this syndrome is poorly characterized. We describe an 18 month-old male who was born at 36 weeks via C-section after an uneventful pregnancy. During the newborn period, upon examination, he was found to have a right-sided cleft lip and cleft palate, hypertelorism, strabismus, mildly over-folded ears with cupping and hypothyroidism. Cardiovascular examination was consistent with the diagnosis of severe aortic coarctation, which was confirmed by echocardiogram. Neurologic evaluation at 18 months revealed a hypotonic infant with delayed acquisition of motor milestones. Thus, the main clinical features of our patient are related to CNS development, facial/palate/lip development, thyroid development and aortic arch formation. Cytogenetic analysis showed a duplication of proximal 20q: 46,XY,dup(20)(q11.2q13.1). FISH analysis with a wcp20 probe confirmed the chromosome 20 origin of the extra material. Parental karyotypes were normal indicating a de novo origin for the duplication. Array CGH confirmed the duplication of proximal 20q and further allowed quantitation of the size of the duplication. The clinical, cytogenetic and molecular characterization of this patient presented here provides the first detailed description of this syndrome.

**Case report: molecular characterization of the terminal end deletion in an inversion duplication of the short arm of the chromosome 8.** *S. Bourthoumieu<sup>1</sup>, M.C. Vincent<sup>1</sup>, P. De Mas<sup>1</sup>, J.Y. Le Tallec<sup>2</sup>, P. Calvas<sup>1</sup>, E. Bieth<sup>1</sup>.* 1) Medical Genetics, hopital Purpan, Toulouse, France; 2) hopital des enfants, Toulouse, France.

Inversion duplications of the short arm of chromosome 8 (inv dup 8p) are considered as one of the more common cytogenetic abnormalities. This rearrangement usually results in a well defined clinical syndrome including hypotonia in children, mental retardation, agenesis of the corpus callosum, dysmorphic feature and occasionally congenital heart defect. Because terminal 8p deletions alone are usually associated with no or mild phenotypic effects, it has been thought that clinical features of the inv dup 8p syndrome are mainly due to the duplicated segment and poorly from the loss of 8p subtelomeric sequences. However, some reports suggest that differences in mental retardation severity or association with organ defects might be explained by partial monosomy for the telomeric segment. We report on a 21 month old girl who was referred for developmental delay . She had agenesis of the corpus callosum and morphological abnormalities including microcephaly, supernumerary nipples and deep plantar creases. Echocardiogram failed to detect any congenital heart defects. Cytogenetic analysis of her peripheral blood using R and G banding techniques revealed an inverted duplication 8p associated with a partial monosomy 8pter [(46,XX,inv dup(8)(p23p12)del(8)(pterp23)]. FISH using a centromere-specific probe showed hybridization at the terminal end of the short arm of the duplicated chromosome 8. These data are consistent with a proposed mechanism of inv dup 8p rearrangement which involves the formation of a dicentric intermediate (Floridia et al.). Molecular studies using microsatellite markers covering the entire chromosome 8 allowed us to obtain a fine characterization of the 8p23.18pter deletion in this patient. Our findings provide clarification on the phenotypic contribution of the concomitant terminal deletion in inverted duplication of chromosome 8p. We discuss more particularly the molecular involvement of this deletion in the generation of congenital heart defects.

**46,XX/46,XY mosaicism and not chimerism in a hermaphrodite infant confirmed by molecular analysis.** *I.K. Gadi<sup>1</sup>, P.R. Papenhausen<sup>1</sup>, P.D. Singh-Kahlon<sup>1</sup>, L. Wisniewski<sup>1</sup>, P.N. Mowrey<sup>1</sup>, P. Jayakar<sup>2</sup>, B. Williford<sup>1</sup>, C. Schott<sup>1</sup>, J.H. Tepperberg<sup>1</sup>.* 1) Dept Cytogenetics, Laboratory Corp of America, Res Triangle Pk, NC; 2) Division of Genetics, Miami Childrens Hospital, Miami,FL.

To our knowledge, this is the second reported case of a true hermaphrodite confirmed by molecular methods. An infant born to a 27 year old mother by normal vaginal delivery was referred for peripheral blood cytogenetic analysis because of ambiguous genitalia. The phallus length was 1.2cm with a normal urethral opening and a fistula at the base. The right testicle was palpable in the scrotal fold but there was no evidence of the left testicle. Ultrasound examination showed urogenital sinus, vagina and uterine structures. Cytogenetic analysis of 50 cells revealed 33 cells with a normal female chromosome complement and 17 cells with a normal male chromosome complement. Fetal derivation of the blood was confirmed by hemoglobin isoelectric focusing. Molecular analysis of polymorphic microsatellite loci from twelve different chromosomes revealed a maternal and a paternal allele at all informative loci. Each of these highly polymorphic loci showed a maximum of two alleles consistent with a single zygotic origin of the infant. Therefore, genotype identical 46, XX and 46, XY cell lines observed in the infant most likely originated by two separate non-disjunctions from a 47, XXY embryo with subsequent loss of XXY cells. Maternal and paternal X chromosome alleles were also apparent at all X chromosome loci examined that will rule out origin of 46,XX cells from 46,XY cells by loss of Y chromosome and gain of X chromosome. These results are consistent with true mosaicism in the hermaphrodite infant rather than chimera.

**Utility of an Interphase FISH Panel to Screen for Common Chromosome Aneuploidies in 178 Spontaneous Miscarriages.** *B.M. Shearer, R.A. Mota, K.R. Hammer, S.M. Jalal, R.P. Ketterling.* Cytogenetics, Mayo Clinic, Rochester, MN.

Approximately 50% of spontaneous miscarriages, which occur during the first trimester of pregnancy, are due to numeric chromosome abnormalities. The most common chromosome anomalies observed include monosomy X, triploidy, and trisomies of chromosomes 13, 16, 18, 21 and 22. Unfortunately, approximately 20% of miscarriage tissues fail to produce a successful chromosome result due to a lack of viable dividing cells. Since interphase fluorescence in situ hybridization (FISH) analysis does not require dividing cells, we utilized a FISH panel of 7 probes (chromosomes 13, 16, 18, 21, 22, X and Y) to analyze each tissue for which standard chromosome analysis was unsuccessful. To implement this strategy, all miscarriage tissues referred for cytogenetic testing were simultaneously processed for both chromosome analysis (cultured) and FISH analysis (uncultured). A total of 178 miscarriage tissues were analyzed by the interphase FISH panel. The overall abnormality rate using the seven probe strategy was 22% (39 specimens) which included 3 samples with trisomy 13 (1.7%), 4 samples with trisomy 16 (2.2%), 5 samples with trisomy 18 (2.8%), 5 samples with trisomy 21 (2.8%), 3 samples with trisomy 22 (1.7%), 13 samples with triploidy (7.3%) and 6 samples with monosomy X (3.4%). Normal results were obtained for 122 samples (68%) while 17 samples (10%) were unsuccessful by FISH. The results of this study strongly suggest that FISH panel testing has proven efficacy for the detection of common chromosome aneuploidies associated with spontaneous miscarriages.

**45,X/46,XY mosaicism confined to amnion in a stillborn 46,XY male with IUGR.** *J. Bourgeois, J. Xu.* Pathology & Molecular Medicine, McMaster University, Hamilton, ON, Canada.

Confined placental mosaicism is found in 1-2% of CVS cytogenetics cases and is associated with an increased risk of spontaneous abortion, growth retardation, fetal demise, or uniparental disomy. We report a rare case with mosaic X/XY amnion in an unexplained stillbirth. This male was delivered at 25 weeks gestational age (GA) to a 40 year old G1P2 woman. The pregnancy was complicated by intrauterine growth restriction, oligohydramnios and intrauterine demise. Following delivery, the stillbirth showed symmetric growth restriction with measurements consistent with 22-23 weeks GA and minor external anomalies including low set ears and overlapping toes. The placenta was grossly and microscopically normal. A normal male karyotype 46,XY was found in all 14 in situ colonies from the amniocentesis at 15.2 weeks GA and 20 metaphases in cultured peripheral blood. However, mosaic 45,X[15]/46,XY[15] was observed in cultured amnion tissue taken at the time of fetopsy. While it is well documented that 90-95% of prenatally diagnosed mosaic X/XY cases give rise to a phenotypically normal live born male baby, our case seems to suggest that mosaic X/XY in amnion may be detrimental to fetal development. More cases of this kind are needed to confirm this hypothesis. This case emphasizes pathological significance of cytogenetics investigation of placental tissues in addition to routine studies of prenatal and fetal samples in a karyotypically normal stillbirth.

**FISH: an effective and less costly technique for genetic evaluation of products of conception in pregnancy losses.**

*A. Amiel<sup>1,3</sup>, M. Liberman<sup>3</sup>, M. Pomeranz<sup>2</sup>, A. Fishman<sup>2,3</sup>, M. Fejgin<sup>1,2,3</sup>.* 1) Genetic Institute, Kfar-Saba, Israel; 2) Department of Obstetrics & Gynecology, Meir Hospital, Israel; 3) Sackler School of Medicine, Tel Aviv University, Israel.

Fetal chromosomal abnormalities account for about 50 to 70% of first trimester pregnancy losses. Most of these are numerical abnormalities (86%) and the minority are structural abnormalities (6%) and mosaicism (8%). Since conventional cytogenetic analysis of the products of conception is costly and time consuming, it is not performed routinely, leaving a question mark on the cause of miscarriage. FISH technique for common numerical aberrations may uncover a major part of the genetic causes for pregnancy loss at a fraction of the cost. Also, bacterial contamination is not an issue. We evaluated the effectiveness of FISH in detection of numerical abnormalities in 59 cases of spontaneous abortion and used 61 electively terminated pregnancies as controls. The probes used were 13,21,16,18, X and Y which should identify in about 75% of the common aberrations in the spontaneous miscarriage. In 55.9% of the spontaneous miscarriage numerical aberrations were diagnosed compared to 8.2% in the cases of elective pregnancy termination ( $p < 10^{-5}$ ). The most common aberrations were: triploidy (16.9%), sex chromosome aberrations (13.6%), trisomy 21 (8.5%), trisomy 16 (6.8%), trisomy 13 (6.8%), trisomy 18 (3.4%). There risk of aneuploidy was in direct correlation to maternal age ( $p < 0.005$ ). We find FISH to be a reliable and rapid tool in detecting the frequent aneuploidies in cases of pregnancy loss. Based on the low cost of this test and the utmost importance of understanding the cause of pregnancy loss it may be feasible to offer it in all cases of pregnancy. Furthermore, it probably should replace the routine histological examination.

**Partial distal trisomy 12q in a father and daughter.** *L. Bao, E.K. Schorry.* Division and Program of Human Genetics, Cincinnati Child Hosp Med Ctr, Cincinnati, OH.

Partial trisomy 12q (q24.31qtel) was found in a girl and her father. The proband was a 19 month old white female referred for developmental delays and dysmorphic features. On examination, she had normal growth parameters, curly and kinky short hair, prominent forehead with frontal bossing, simplified helices of the ears, and a small mouth with thin lips. Both of her parents were mentally retarded. Her father was felt to function in the borderline or mild range of mental retardation, and had macrocephaly, broad forehead, simplified helices of the ears, and small mouth with thin lips. The mother had a more significant degree of mental retardation, and had dysmorphic features very similar to the daughter, with curly and kinky short hair, small deeply set eyes, and small mouth with thin lips. Standard and molecular cytogenetic analysis revealed that both the girl and her father had an unbalanced 12;16 translocation with a der(16)t(12;16)(q24.31;q24.3) while the mother had a normal karyotype. Most of the daughter's dysmorphic features were felt to be related to a possible dominantly inherited syndrome from the mother. Features in common with both the daughter and father which might be attributed to trisomy 12q24.31-q telomere include mild developmental delays, borderline or mild mental retardation, broad forehead, and small mouth with thin lips. Few partial trisomy 12q cases have been reported, and to our knowledge, this is the first reported case of familial transmission of this abnormality.

**Molecular and phenotypic characterization of 9p23-p24.3 deletions.** *S. Biton, C. Crowe, L. Christ, M. Eichenmiller, M. Graff, S. Schwartz.* Genetics, case western reserve universit, cleveland, OH.

The 9p deletion syndrome is a well-established deletion syndrome, but with a variable phenotype. A deletion involving a region on 9p22.3 (the "critical region") has been specifically implicated in the presence of hallmark clinical manifestations detected at birth including: trigonocephaly, upslanting palpebral fissures, wide flat nasal bridge, arched eyebrows, hypertelorism, long philtrum, anteverted nostrils, micrognathia, short and broad neck, long middle phalanges, and an increased number of dermatoglyphic whorls. To date, no study has investigated the correlation between karyotype and phenotypic presentation among individuals with smaller deletions, i.e., those deletions distal to and not including the delineated "critical region". This study's purpose was to better correlate phenotypic to karyotypic findings of patients that display abnormalities due to a deletion of the terminal region of 9p (outside the "critical region"). Fourteen patients with deletions of 9p23-p24.3, encompassing a region of approximately 15 Mb, represent the largest cohort of patients to be systematically studied. High resolution chromosome analysis, microsatellite and molecular cytogenetic (FISH) analysis were utilized to define the breakpoints, the extent of the deletions, and their parental origin. The results from this study yielded several interesting and important findings: 1) a lack of specific hotspots for breakpoint occurrence; 2) a statistically significant number of paternally derived deletions; 3) a preliminary phenotypic map of the investigated region; 4) delineation that all of the patients presented with hypotonia, developmental delay and behavioral abnormalities regardless of the deletion size; and 5) all of these patients had similar findings to patients with "classical" 9p deletion syndrome but with no dysmorphic facial features. Establishment of phenotypic maps should be useful to genetic counselors and medical geneticists both prenatally and postnatally. The results of this study will assist in diagnosis, provision of more accurate diagnosis and in facilitation of decision making.

**Cytogenetic studies of human lymphocytes exposed to sodium pertechnetate and its reducing agent stannous chloride, in vitro.** *I.P. Aranha*<sup>1</sup>, *V.R. Bomfim*<sup>2</sup>. 1) Inst Biol, PHLC S-525-6, Univ Estado Rio de Janeiro, Rio de Janeiro, RJ, Brasil; 2) Sociedade Universitária Augusto Motta, Rio de Janeiro, RJ, Brasil.

Technetium-99m (<sup>99m</sup>Tc) is a marker for molecules and cells widely used in the detection of inflammatory sites as well as in the diagnosis of the rejection of transplanted tissues. Its efficiency is increased by a reducing agent, stannous chloride (SnCl<sub>2</sub>·2H<sub>2</sub>O). Previous work in our laboratory has shown that the use of the reducing agent in the culture of whole blood lymphocytes neither decreased the mitotic rate nor caused any numerical or structural chromosomal abnormality. The goal of the present work was to observe if sodium pertechnetate combined with its reducing agent might cause chromosomal alterations in human lymphocytes in vitro. Peripheral whole blood cells, collected from healthy donors, 18 to 30 years old, were incubated at 37°C for 48h in the presence of <sup>99m</sup>Tc (3.7 MBq/100l) combined to SnCl<sub>2</sub>·2H<sub>2</sub>O (12g/ml). Cells not exposed to the radionuclide served as control for the experiment. Chromosomes were stained with Giemsa Gurr (2%) and observed under optical microscope. In the test group, 1469 metaphases were studied; 1073 were normal and 396 carried chromosomal alterations (242 gaps and 267 breaks). In the control group, 1102 metaphases were observed, being 1042 normal and 60 abnormal (42 gaps and 30 breaks). Our results were extremely significant ( $\chi^2$  with Yates correction = 198.24 p0.0001). They suggest that sodium pertechnetate combined with stannous chloride was responsible for the chromosomal alterations observed.

**Double balanced translocations t(5;22) and t(6;8) in a woman with recurrent miscarriages.** *Z. Chen<sup>1</sup>, D. Tang<sup>2</sup>, C.N. Huang<sup>1</sup>, X.M. Song<sup>1</sup>, J. Xu<sup>3,4</sup>.* 1) Medical Genetics, Zhongshan Medical College, Sun Yat-sen University, Guangzhou, PR China; 2) Medicine; 3) Pathology and Molecular Medicine; 4) Lab Medicine, Hamilton Health Sciences and McMaster University, ON, Canada.

Double balanced translocations are rare; only a few cases are documented in literature. Some of which are associated with clinical complications, possibly a result of submicroscopic aberrations in the breakpoints. Others are associated with a normal phenotype, but with significantly increased reproductive risk of miscarriages, stillbirths or liveborn children with anomalies. Here we present a case of a 27-year-old and phenotypically normal woman who had cytogenetics analysis done because of three recognized miscarriages over the period of 5 years and no liveborn children. The gestational age for the miscarriages is ~60 days for the 1st, ~40 days for the 2nd, and ~60 days for the 3rd. Routine G-banding analysis of the cultured peripheral bloods of the couple revealed that the husband had a normal male karyotype 46,XY and the woman had two independent and apparently balanced translocations 46,XX, t(5;22)(q15;q11),t(6;8)(q21;q22). The origin of the translocations remains unknown because the parents are not available for karyotyping. The double translocations in our patient are likely the cause of her repeated abortions.

**Telomeric FISH screening in population with mental retardation or reproductive failure.** *D. Giardino<sup>1</sup>, P. Finelli<sup>1,2</sup>, C. Corti<sup>1</sup>, G. Gottardi<sup>1</sup>, M.T. Bonati<sup>1,3</sup>, F. Natacci<sup>3,4</sup>, M. Della Monica<sup>5</sup>, G. Scarano<sup>5</sup>, L. Larizza<sup>1,2</sup>.* 1) Lab. Citogenetica, Istituto Auxologico, Milan, Italy; 2) Dip. Biologia e Genetica per le Scienze Mediche, Università di Milano, Milan, Italy; 3) Fondazione Istituto Sacra Famiglia, Cesano Boscone, Italy; 4) Servizio di Genetica Medica, ICP, Milano, Italy; 5) U.O.C. di Genetica Medica, A.O.R.N. G. Rummo, Benevento, Italy.

Subtelomeric chromosome rearrangements are a cause of mild to moderate mental retardation (MR), particularly when associated with dysmorphism. They could also account for recurrent miscarriages or reproductive failures. Sets of commercially available subtelomeric fluorescent DNA probes have been used to detect subtelomeric anomalies in patients with MR, with or without dysmorphic features (DF), and individuals with a history of multiple miscarriages (MM) and/or reproductive failures (RF). All the patients were karyotypically normal by Q-banding technique. The group of subjects with MR was also negative for FRAXA and FRAXE expansion. 108 cases were analyzed, 73 with MR/DF and 35 with MM/RF. FISH was performed with Chromoprobe Multiprobe T System (Cytocell) and then with locus specific probes to characterize the observed abnormalities. In the MR group 10/73 anomalies were found, 7 were inherited from a phenotypically normal parent and interpreted as benign polymorphism, 2 were derivative chromosomes consequent to a parental balanced translocation and one was a 22qter cryptic deletion. Both the derivative chromosome determine a 2q subtelomeric deletion, with a concurrent trisomy of 16q and 6q, respectively. The extent of the 2q deleted regions in the two patients was determined by targeted FISH with BAC clones. The 2q deletion interval in one of our patients is smaller than that previously defined for Albright Hereditary Osteodystrophy-like syndrome, with the centromeric boundary moved >1.0 Mb towards the 2q telomere. No subtelomeric abnormalities were found in the MM/RF group, while two polymorphisms were detected. According to our experience telomeric FISH screening appears to be a useful tool for diagnostic and prognostic purposes in children with MR, but it does not seem such a targeted approach for couples with MM/RF.

**Definition of chromosomal abnormalities in children with multiple congenital anomalies, dysmorphic features and mental retardation using spectral karyotyping (SKY).** *S. Ben Shachar<sup>1</sup>, Y. Yaron<sup>1,2</sup>, D. Kunig<sup>1</sup>, M. Goldstein<sup>1,2</sup>, N. Furman<sup>1</sup>, R. Shomrat<sup>1</sup>, A. Orr-Urtreger<sup>1,2</sup>.* 1) Genetic Institute, Tel Aviv Sourasky Medical Cent, Tel Aviv, Israel; 2) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Some chromosomal aberrations cannot be elucidated by standard cytogenetic techniques. Spectral karyotyping (SKY) is a molecular cytogenetic technique that allows the simultaneous and rapid identification of all 24 chromosomes in different colors. We hereby describe the use of spectral karyotyping technique in the identification of chromosomal aberrations that could not be interpreted by standard cytogenetic techniques. The study included four children with psychomotor delay and dysmorphic features. G-band staining revealed chromosomal aberrations in all cases. Spectral karyotyping analysis was performed to further define the nature of the abnormality. Patient 1, a 2-year old girl with recurrent infections, psychomotor delay and dysmorphic features was found to have additional material on the long arm of chromosome 11. Using SKY analysis we determined that this was a 11q24-25 duplication. Patient 2, a 3-year old girl with development delay, hemiparesis and dysmorphic features had an extra structurally abnormal chromosome (ESAC). The origin of this ESAC could not be determined by G-band staining. Spectral karyotyping demonstrated that the origin of this ESAC was chromosome 5. The chromosomal G-band analysis of patient 3, a 4-year old boy with psychomotor delay and dysmorphic features, showed an additional chromosomal material on chromosome 3. It was proved to be a 3q duplication by spectral karyotyping analysis. Patient 4 was a neonate with multi organ defects and dysmorphic features. A combination of SKY and FISH techniques demonstrated partial trisomy of 1qter and partial 11qter monosomy. We conclude that spectral karyotyping is a powerful adjunct tool to standard cytogenetic analysis in patients with multiple congenital anomalies, dysmorphic features and mental retardation.

**The molecular basis of terminal deletions.** *B.C. Ballif<sup>1</sup>, W. Yu<sup>1</sup>, C.D. Kashork<sup>1</sup>, C.A. Shaw<sup>1</sup>, M. Gajecka<sup>2</sup>, H.A. Heilstedt<sup>1</sup>, L.G. Shaffer<sup>1,2,3</sup>.* 1) Dept Molecular & Human Genet, Baylor Col Medicine, Houston, TX; 2) Health Res & Edu Ctr, Washington State Univ, Spokane; 3) Sacred Heart Med Ctr, Spokane.

Terminal deletions are one of the most commonly observed chromosome abnormalities and result in several well-known mental retardation syndromes. However, the molecular mechanisms that generate and stabilize terminal deletions are poorly understood. We developed a contig of the most distal 10.5 Mb of 1p36 and characterized the deletions in 60 subjects with monosomy 1p36. Deletion sizes varied widely, with no single common breakpoint. Terminal deletions, interstitial deletions, complex rearrangements, and derivative chromosomes were identified. Ten rearrangements of 1p36 were examined at the DNA sequence level. Seven were apparently terminal deletions and three were known derivative chromosomes, der(1)t(1;1)(p36;q44). Sequence analysis at the junctions indicated that one was a pure terminal truncation stabilized by telomeric repeat sequences. Analysis of two cell lines identified terminal deletions associated with cryptic interrupted inverted duplications. These junctions are identical in structure to those found in cell lines that have gone through breakage-fusion-bridge cycles where uncapped sister chromatids are fused by non-homologous end joining. In four subject cell lines, the breakpoints occurred in repetitive DNA sequence elements (LINEs, SINEs, etc.). Analysis of the der(1) chromosomes junctions did not identify low-copy repeats in the regions, suggesting that nonallelic homologous recombination did not mediate these rearrangements. These data are consistent with the mechanism of telomere capture by break-induced replication at the site of a double-strand break generating nonreciprocal translocations from terminally deleted chromosomes. The results of the molecular analyses of 10 terminal deletions of 1p36 are strikingly similar to recent observations in model systems where a variety of mechanisms compete to repair a double-strand break near a telomere and stabilize the end of a broken chromosome.

**Two interstitial deletions in a subject with a complex chromosomal rearrangement of 1p36.** *M. Gajicka<sup>1</sup>, B.C. Ballif<sup>2</sup>, S.K. Shapira<sup>3</sup>, W. Yu<sup>2</sup>, L.G. Shaffer<sup>1,2,4</sup>*. 1) Health Res & Edu Ctr, Washington State Univ, Spokane; 2) Dept Molecular & Human Genetics, Baylor Col Med, Houston, TX; 3) Dept of Pediatrics, Univ of Texas Health Sci Ctr, San Antonio; 4) Sacred Heart Med Ctr, Spokane, WA.

Although terminal deletions of 1p36 are relatively common (~1 in 5,000 live births), interstitial deletions of 1p are less frequent, and little is known about their mechanisms of formation. We studied a subject with two interstitial deletions within the 1p36 region that are present on the same chromosome 1. The complex rearrangement was detected using FISH, microsatellite analysis, and microarray-based comparative genomic hybridization (array CGH). Array CGH was performed using a 1p36 microarray constructed from a contig that consists of 97 large-insert BAC clones spanning 10.5 Mb of the most distal region of 1p36 and 41 subtelomere clones from all chromosomes except the acrocentric short arms. Four breakpoints in the subject's cell line were identified. Additionally, the cell line was fused to HPRT-deficient RJK88 hamster cells to generate somatic cell hybrids. Hybrid colonies containing the chromosome 1 with the 1p36 interstitial deletions segregated from the normal chromosome 1 homologue were identified. Each junction was further refined by PCR and STS marker walking on hybrid DNA. By this approach, we sequenced the proximal interstitial deletion junction. Sequence analysis of the junction revealed that each breakpoint is located within a repetitive DNA sequence element. Continued investigation of the distal deletion junction of this subject and other complex rearrangements of 1p36 may uncover the mechanism(s) resulting in interstitial deletions and rearrangements of telomeric regions.

**Unusual clinical presentation of a patient with ring chromosome 4: cytogenetic molecular characterization.** *D. Concolino<sup>1</sup>, R. Apa<sup>1</sup>, R. De Marco<sup>1</sup>, M.A. Iembo<sup>1</sup>, E. Rossi<sup>2</sup>, V. Pascale<sup>3</sup>, P. Strisciuglio<sup>1</sup>.* 1) Department of Pediatrics, University "Magna Graecia", Catanzaro, Italy; 2) Istituto di Patologia Umana ed Ereditaria, Universit di Pavia, Pavia, Italy; 3) Divisione di Neonatologia, Ospedale Pugliese, Catanzaro, Italy.

The ring chromosomes are cause of malformations, mental and growth retardation. The presence of a ring chromosome 4 is a rare event and usually shows a predominant loss of distal 4p including the Wolf-Hirschhorn region (WHS). Few cases of molecular characterization of ring 4 were reported in literature. We describe a newborn with a case with r(4) who presented without features of WHS; she had pre-postnatal growth retardation, microcephaly, minor dysmorphic signs and renal malformations. Cytogenetic studies were performed by QFQ and GTG banded metaphases from synchronised peripheral blood lymphocyte cultures using standard procedures. Probands karyotype was interpreted as 46,XX,r(4)(p16q35) in all metaphases observed. Parental karyotypes were normal. FISH analysis with telomeric 4p and 4q probes failed to detect 4q signal but there was no 4p ter fluorescent signal. A DNA probe corresponding to the Wolf-Hirschhorn region showed two copies of the signal. The ring breakpoints identified by FISH analysis were 46,XX,r(4)(p16.3;q35.2).ish r(4)(4ptel-,WHS+,4qtel+). The phenotype of our case seems different from previous patients for the presence of renal malformations and absence of psychomotor developmental delay probably due to a small deletion on the region 4p.

**Molecular and cytogenetic characterization of breakpoints by FISH in 14 cases of isodicentric Y chromosomes.**

*M. Beaulieu Bergeron*<sup>1,4</sup>, *M. DesGroseilliers*<sup>1,4</sup>, *R. Fetni*<sup>5</sup>, *P. Brochu*<sup>3</sup>, *E. Lemyre*<sup>2,4</sup>, *N. Lemieux*<sup>1,3,4</sup>. 1) Département de Pathologie et biologie cellulaire, Université de Montréal, Montréal, Québec, Canada; 2) Département de Pédiatrie; 3) Département de Pathologie et; 4) Centre de recherche de l'Hôpital Sainte-Justine, Montréal, Québec, Canada; 5) Department of Pathology, McGill University Health Center, Montreal, Quebec, Canada.

Infrastructural rearrangements of the Y chromosome are not rare, especially isodicentrics. These idic(Y) are greatly unstable during meiosis and mitosis, and patients having such rearrangement often present mosaicism. Most severe phenotypes, including Turner syndrome and sex reversal, are almost exclusively seen in females bearing an idic(Y). In male cases, clinical implications often include azoospermia and infertility. We present the FISH results of 14 cases of idic(Y) including 6 males, 7 females and one individual with sexual ambiguity. FISH analyses were performed using probes SRY (Yp11.3), 91H4.5 (Yp11.2), DYZ1 (Yq12), DYZ3 (Y centromere) and WCPY (whole chromosome painting of the Y). BACs (Bacterial Artificial Chromosomes) were used in some cases in order to refine the molecular breakpoints. Breakpoints were all localized in the Yq11.2-Yq12 region except for one case, a female with a 46,X,idic(Y)(p11.2)/45,X SRY- karyotype. Except for the latter and a male with a 47,X,idic(Y)(q12)x2/45,X karyotype, all other cases were negative for heterochromatin. Of the 14 cases, one male and 5 female patients show a 45,X cell line in addition to the idic(Y). Furthermore, 2 males show a 46,X,idic(Y)(q11.21) chromosomal constitution without any mosaicism, while 2 females, the individual with sexual ambiguity and 2 males were shown to have a deleted Y chromosome or two idic(Y) in addition to the idic(Y) cell line. When present, the 45,X cell line was found in variable proportions. Finally, one male presents a 47,XY,idic(Y)(q11.21)/46,XY karyotype. Thus, clinical implications of idic(Y) are greatly variable and depend not only on the percentage of cells showing a 45,X constitution or structurally abnormal Y chromosomes, but also on the sexual differentiation, since only females generally show severe clinical problems. *Supported by RMGA-FRSQ.*

**Ring 2 chromosome case report and cell cycle studies.** A. Corona-Rivera<sup>1,2,5</sup>, L. Bobadilla-Morales<sup>1,5</sup>, S. Mendoza-Becerra<sup>1</sup>, F. Carbajal-López<sup>1</sup>, P. Barros-Núñez<sup>4</sup>, J.R. Corona-Rivera<sup>1,3,5</sup>, *Cuerpo Académico Genética Médica y Clínica, CUCS, U. de G.* 1) Laboratorio de Genética Humana, Universidad de Guadalajara, CUCS, Guadalajara, Jalisco, México; 2) Unidad de Citogenética, OPD Hospital Civil de Belén, Guadalajara, Jal., México; 3) OPD, Hospital Civil Juan I. Menchaca, Guadalajara, Jal., México; 4) CIBO, IMSS, Guadalajara, Jal., México; 5) Doctorado en Genética Humana, CUCS, U. de G., México.

We are aware of ten previously reported cases of ring 2 chromosome (r(2)). Ring chromosomes without an important deletion share a common pattern of phenotypic anomalies, which occurs independently of the chromosome involved, named as General ring syndrome. Such common pattern exhibit Intrauterine Growth Retardation, postnatal growth failure, and a mild-to-moderate mental retardation. We report a patient concordant with General ring syndrome features, whose also showed severe microcephaly, obesity, hyperphagia, recurrent medial otitis and hyperkinesia. At birth, routine cytogenetic analysis showed karyotype 46,XY,r(2)(p25;q37) in all cells. At 6 years old, subsequent kayotypes showed mosaicism with increasing appearance, up to 30%, of normal cells and low frequency of ring variants such as dicentric or double rings, and tetraploidy. It has been proposed that the normal occurrence of sister chromatid exchanges within a ring constantly produces further chromosomal abnormalities resulting in a high cellular death rate in vivo and in vitro and to end with a decrease in the total number of viable cells present at any given interval of proliferation. Cellular proliferation kinetics was measured in our patient by differential sister chromatid stain evidencing lower proliferation rate in cells bearing r(2). Besides, r(2) frequency gradually decreased in three different culture times. Observed r(2) instability and clinical findings agree with the fact that ring size and ring behavior, rather than its genetic content, are involved and may be associated with the growth deficiency.

**CYTOGENETIC FINDINGS IN 780 COUPLES WITH RECURRENT ABORTION.** *R. Baez-Reyes, JL. Saucedo-Hernandez, DG. Mayen- Molina.* Genetica, Inst. Nac. de Perinatologia, Mexico, Edo.Mex.

Recurrent gestational loss is a very important reproductive health problem in our country since it affects 1% of the couples. It has an heterogeneous etiology, which means that the geneticists must face medical and psycho-social dilemmas and problems. It has been estimated that about 50 to 60% of the first trimester spontaneous abortions undergo chromosome anomalies. The latter may be either inherited or de novo. A sample of 1560 cytogenetic studies from 780 couples with two or more spontaneous abortions were analyzed from January, 1984 to June, 2003. Peripheral blood lymphocytes were cultured after the clinical evaluation of the patients. GTG bands were obtained and fifteen metaphases from two primary cultures were analyzed. If an altered cell was found, the mitotic count was increased up to 100 metaphases. The frequency of chromosome anomalies per couple observed in our sample was 7.3 %. Balanced reciprocal translocations ( 2.69 % ), robertsonian translocations ( 1.02 % ), sex chromosome mosaicism ( 2.43 % ), autosomal mosaicism ( 0.76 % ) and inversions ( 0.38 % ) were the alterations found. International papers report 4.8-5.5% of chromosome alterations in couples with recurrent abortions. The cytogenetic study in peripheral blood lymphocytes, as well as in fetal tissues and placental annexa, must be considered as a diagnostic tool in the evaluation of patients who have undergone one or more abortions in order to be able to give appropriate information and opportune genetic counseling. The forementioned will create the possibility to offer a better medical and psychological attention, as well as a prenatal diagnosis for the future pregnancies. On the other hand, this will help to detect fetal anomalies in good time.

**A de novo intrachromosomal inverted duplication of chromosome 18 with deletion of subtelomeric region in a newborn with severe congenital anomalies.** *D. Flannery<sup>1</sup>, S.M. DeLaney<sup>1</sup>, A.S. Kulharya<sup>1,2</sup>*. 1) Dept Pediatrics, Sect Genetics, Medical College of Georgia, Augusta, GA; 2) Dept Pathology, Medical College of Georgia, Augusta, GA.

Duplication deletion of chromosome 18 is rare; most result as recombinants from an inverted chromosome 18 in a carrier parent. Partial trisomy of chromosome 18 resulting from intrachromosomal duplication accompanied by a deletion has not been previously demonstrated. We present a patient with intrachromosomal duplication of chromosome 18 and a submicroscopic deletion of chromosome 18 demonstrated by FISH. The patient was a full term infant delivered to a 33-year old G3P1 mother and 29-year old father. At birth, he was noted to have large anterior fontanelle, hypertelorism, low set ears, wide spaced nipples and an absent kidney. He has bilateral arthrogryposis of the hands and feet. The tympanic membrane was abnormal with a hyper-dense area. At 6 months of age he underwent fundoplication to correct GE reflux. He is developmentally delayed. Chromosome analysis demonstrated additional material on the long arm of chromosome 18. FISH with subtelomeric probes for the short and long arm of chromosome 18 and whole chromosome paint (WCP) was performed. The hybridization pattern with WCP 18 is consistent with duplication of 18. The subtelomeric probe for 18p shows a normal hybridization pattern while the signal for 18q subtelomeric probe is deleted on the abnormal 18. The karyotype is: 46,XY,add(18)(q23). ish dup(18)(pter->q23::q23->q21.3)(wcp18+),del(18)(q23)(D18S1390-) The terminal deletion at 18q involves the region commonly deleted in the 18q-syndrome which has an incidence of 1/40,000 and a highly variable phenotype with mental retardation, short stature and foot anomalies as common features. Two critical regions of partial duplications of chromosome 18 have been identified that produce phenotypic features of trisomy 18. These regions lie within 18q12.1-18q21.2 and 18q21.3->qter. Our patient has duplication of the second of these critical regions and reflects some features of trisomy 18. This patient confirms the recent findings that intrachromosomal inverted duplications are accompanied by deletions.

**De Novo direct duplication of chromosome 11 (q13-qter) associated with dysmorphism - A case report and review.** *S. Abulhasan<sup>1</sup>, D.S. Krishna Murthy<sup>2</sup>, R. Al-Dabbus<sup>3</sup>, M. Abdul Rasool<sup>1</sup>, S.A. Al-Awadi<sup>3</sup>.* 1) MOLECULAR CYTOGENETICS and Cytogenetics laboratory, MEDICAL GENETICS CENTER, Kuwait; 2) Cytogenetics Laboratory,; 3) MEDICAL GENETICS CENTER, Kuwait.

Duplication of autosomal segments lead to less severe clinical manifestations and handicap than deletion of the same segments, some deletions are even lethal. Partial trisomy for autosomal segments result from translocations (de novo/inherited). However, segmental direct duplications are very rare. We report here a newborn female with minor dysmorphic features, congenital heart defect (PDA), bossing forehead, short nose, low set ears, prominent philtrum, Ultrasonography scan of skull and abdomen was normal. There is no consanguinity and family history is unremarkable. Proband's 10 sisters and a brother are all normal. Chromosome analysis of the peripheral blood metaphase chromosomes using G-banding technique showed an apparently large segment of chromosome 11 (duplication of 11q13-q23). Karyotype of the parents were normal. FISH studies were carried out using different probes specific for chromosome 11 (WCP11, CyclinD1 locus specific and Tel11qter), confirmed direct duplication of chromosome 11(q11-q23). In most of the patients reported the partial trisomy is familial, resulting from balanced translocation heterozygote carrier parent, de novo cases are very rare. The recurrence risk and the clinical manifestations is compared with other similar cases of trisomy 11q.

**Prenatal Overgrowth and Mosaic Trisomy 15q25-qter Including the IGF1 Receptor Gene.** *L. Faivre<sup>1</sup>, T. Rousseau<sup>2</sup>, N. Laurent<sup>3</sup>, P. Gosset<sup>4</sup>, C. Thauvin-Robinet<sup>1</sup>, S. Lionnais<sup>2</sup>, P. Callier<sup>5</sup>, P. Khau Van Kien<sup>1</sup>, F. Huet<sup>1</sup>, C. Turleau<sup>4</sup>, P. Sagot<sup>2</sup>, F. Mugneret<sup>5</sup>.* 1) Centre de Genetique, Hopital d'Enfants, Dijon, France; 2) Centre pluridisciplinaire de Medecine Foetale, Maternite du Bocage, Dijon, France; 3) Anatomopathologie, Faculte de Medecine, Dijon, France; 4) Cytogenetique, Hopital Necker-Enfants Malades, Paris, France; 5) Cytogenetique, CHU Le Bocage, Dijon, France.

Although chromosome imbalances are commonly associated with growth failure, the number of chromosomal abnormalities associated with overgrowth have started to emerge. In particular, todate, 12 cases of trisomy 15qter have been associated with overgrowth. Here we report on a male foetus presenting with overgrowth and additional material on the short arm of one of the chromosome 15 in 12% of lymphocytes and 50% of amniotic cells. Cytogenetic studies were indicated for elevated maternal age. Parents karyotypes were normal, indicating a de novo origin for this unbalanced rearrangement. Complementary studies using cytogenetic and FISH studies showed that this additional material resulted in a 15q25-qter trisomy and confirmed the presence of three copies of the insulin-like growth factor 1 receptor (IGF1R) gene, included in the trisomic region. Autopsy performed after termination of pregnancy revealed isolated overgrowth (90th centile) and absence of visceral malformations. The possible mechanisms and origins for the formation of this mosaic pure trisomy are complex. The present observation emphasises the hypothesis that the overgrowth phenotype, frequently reported in patients with a trisomic segment involving the 15q26 region, might be causally related to a dosage effect of the IGF1R gene, as well as the importance of chromosome analysis in patients with overgrowth. It also confirms that overgrowth is of prenatal onset in those observations. The present observation raise the question of the indication of a fetal karyotype in prenatal overgrowth when a maternal origin has been ruled out.

**Two cases of partial trisomy 21 resulting from a translocation involving chromosome 21 and a non-acrocentric chromosome initially identified by interphase FISH.** *J.A. Adkins<sup>1</sup>, L. Werhane<sup>1</sup>, D. Gore<sup>2</sup>, D.M. Main<sup>3</sup>, V.S. Tonk<sup>4</sup>, G.N. Wilson<sup>4</sup>, J.B. Ravnani<sup>1</sup>, S.A. Berend<sup>1</sup>.* 1) Genzyme Genetics, Santa Fe, NM; 2) Medical Center of Lewisville, Lewisville, TX; 3) California Pacific Medical Center, San Francisco, CA; 4) Texas Tech Medical Center, Lubbock, TX.

Fluorescence in situ hybridization (FISH) analysis of interphase cells is an effective, rapid method for screening amniocentesis specimens for the common aneuploidies. However, often the chromosome analysis provides additional cytogenetic information that is not obtainable by this test. Together these two tests provide complimentary information. We present two cases of partial trisomy 21 resulting from translocations involving chromosome 21 and a non-acrocentric autosome. Interphase FISH analysis showed three copies of the chromosome 21 probe in each of the two cases. Cytogenetic analysis revealed 46 chromosomes, including a derivative chromosome. Case 1 was referred for an amniocentesis due to abnormal ultrasound findings including agenesis of the corpus callosum, hydrocephalus, a heart defect, and clenched hands. Chromosome analysis showed a derivative chromosome 17. Because the interphase FISH analysis showed three copies of the chromosome 21 probe, it was apparent that the derivative chromosome 17 resulted from a translocation involving the short arm of chromosome 17 and the long arm of chromosome 21. Parental chromosome analyses were not done on this case. Case 2 was referred for an amniocentesis due to an abnormal maternal serum screen with increased risk for Down syndrome. Chromosome analysis showed a derivative chromosome 18. Utilizing the interphase FISH results and the chromosome analysis, it was apparent that the derivative chromosome 18 resulted from a translocation involving the short arm of chromosome 18 and the long arm of chromosome 21. Parental chromosome analyses showed that the mother carried a balanced translocation involving chromosomes 18 and 21. The rearrangement in each of these cases was confirmed by metaphase FISH analysis. These two cases show the importance of following all interphase FISH studies with conventional cytogenetics.

**An unusual case of mosaicism for a cryptic complex inversion duplication 8p and ring (inv dup 8p) demonstrates a novel mechanism of chromosome stabilization.** *J. Chernos*<sup>1,2</sup>, *M. Innes*<sup>2</sup>. 1) Cytogenetics Laboratory, Alberta Children's Hosp, Calgary, AB, Canada; 2) Dept Medical Genetics, University of Calgary, Calgary, AB, Canada.

Inversion duplication 8p is a relatively common chromosome rearrangement with a well-delineated clinical phenotype including severe mental retardation, dysmorphic features, agenesis of the corpus callosum, and malformations of the heart and kidneys. We report an unusual case of mosaicism for an inv dup 8p chromosome present in both a linear and a ring configuration that suggests a novel mechanism for stabilizing the inv dup 8p chromosome. Chromosome studies were initiated at 16 years of age due to agenesis of the corpus callosum, short stature, failure to thrive and dysmorphic features. G-banded karyotype was reported as 46,XY,dup(8)(p23p11.2){27}/46,XY,der(8)r(dup 8)(p23q24.3)[5]. This appeared to be the typical inverted duplication of the 8p21.3p23.3. In theory, no truly terminal deletion exists due to the loss of telomeres and resultant chromosome instability. Telomere healing or telomere capture have been proposed mechanisms to stabilize the terminal inv dup(8) (Giglio, et al, 2001). In this patient, the formation of a ring inv dup 8 represents an alternate stabilization of the rearranged chromosome. In contrast, the linear inv dup 8 must have been stabilized by an another, perhaps unrelated, mechanism. FISH using dual colour chromosome 8 telomere probes yielded surprising results. The linear inv dup 8 lacked the 8p telomere that was replaced by a second copy of the 8q telomere. The ring inv dup 8 similarly, showed no 8p signal but had a signal for 8q. The hypothesized mechanism for stabilizing the inv dup 8 chromosome in this patient is via a ring formation followed by breaking open to produce the linear chromosome that has been stabilizing through capturing some of the 8q telomeric sequences on the short arm. Therefore, the inv dup 8 is actually a cryptic derivative chromosome arising secondary to the ring inv dup 8 configuration.

**Maternal duplication of 15q11-q13 identified in one autism patient during linkage disequilibrium mapping.** *S.L. Christian<sup>1</sup>, L.A. Weiss<sup>2</sup>, H.L. Fritz<sup>1</sup>, C. Lord<sup>3</sup>, N. Akshoomoff<sup>4,6</sup>, E. Courchesne<sup>5,6</sup>, X. Wu<sup>1</sup>, B.L. Leventhal<sup>1,7</sup>, E.H. Cook Jr.<sup>1,2,7</sup>*. 1) Dept Psychiatry, Univ Chicago, Chicago, IL; 2) Dept Human Genetics, Univ Chicago, Chicago, IL; 3) Dept Psychology, Univ Michigan, Ann Arbor, MI; 4) Dept Psychiatry, Univ California, San Diego, La Jolla, CA; 5) Dept Neurosciences, Univ California, San Diego, La Jolla, CA; 6) Childrens Hosp Res Cntr, San Diego, CA; 7) Dept Pediatrics, Univ Chicago, Chicago, IL.

Autistic disorder is a neurodevelopmental disorder that affects ~1 in 500 with a 4:1 male to female ratio. One of the most common genetic mechanisms associated with autism is a ~4 Mb maternal duplication of the 15q11-q13 region that affects ~1-2% of autistic patients. These abnormalities are usually detected using routine cytogenetics and/or FISH. In a study of 92 trios comprised of one autistic child and both parents, linkage disequilibrium (LD) mapping was performed using 116 SNPs across this 4 Mb region. The data were analyzed using software developed by Dr. Wu to identify 3 clusters representing the 1/1, 1/2, and 2/2 genotypes. In one autistic patient the genotyping data for 54 SNPs was found to fall in between otherwise tight heterozygote and homozygote clusters relative to the other data. Examination of the other SNP data on this patient indicated 23 homozygous and 39 heterozygous genotypes. The possibility that this patient had a chromosomal duplication that shifted the SNP data was studied further. Examination of the parental data identified 15 fully informative SNPs that indicated a maternal origin for the shifted results in all cases. Additionally, 5 microsatellites located within this 4 Mb region were analyzed on this patient and both parents. Two markers, D15S541 and strp7, were fully informative and showed the presence of 2 maternal and 1 paternal alleles indicating the presence of a maternal duplication of the 15q11-q13 region and confirming the SNP data. This is the first reported case of a chromosomal duplication identified using SNP mapping data and indicates that careful interpretation of SNP data is needed to prevent overlooking the presence of a chromosomal abnormality.

**Synaptonemal complex analysis in infertile men.** *R. Martin*<sup>1,2</sup>, *F. Sun*<sup>1,2</sup>, *E. Ko*<sup>2</sup>, *L. Barclay*<sup>2</sup>, *G. Kozak*<sup>3</sup>, *K. Trpkov*<sup>4</sup>, *C. Greene*<sup>5</sup>. 1) Department of Medical Genetics, University of Calgary, Calgary, AB, Canada; 2) Department of Genetics, Alberta Children's Hospital, Calgary, AB, Canada; 3) Pathology Department, Rockyview Hospital, Calgary, AB, Canada; 4) Urology Department, Rockyview Hospital, Calgary, AB, Canada; 5) Department of Obstetrics and Gynecology, University of Calgary, Calgary, AB, Canada.

The purpose of this study was to analyze early stages of meiosis in infertile men to determine if any abnormalities in chromosome pairing or recombination could be discovered using new immunocytogenetic techniques. The stages of meiotic prophase, extent of chromosome pairing and the number of recombination foci were compared in men with nonobstructive azoospermia and controls. Immunofluorescence methods were used to identify the synaptonemal complex in various stages of prophase. Antibodies were used to identify the synaptonemal complex (SCP1/SCP3), the centromere (CREST) and sites of recombination (MLH1). Testicular samples from 5 men with nonobstructive azoospermia were studied. Two men had no meiotic cells. One man had an early arrest in meiosis with 13% of cells in leptotene, 87% of cells in zygotene and no pachytene cells. Control donors had 94% of cells in pachytene. One infertile man had a normal number of cells in pachytene, but a decreased number of recombination foci with a mean of 45 (range 2-54), compared to a mean of 48 (range 34-62) in control donors. He also had a large number of cells (25%) with at least one bivalent with no recombination foci, which was rare in controls (4% of cells). The last infertile man had very few meiotic cells (35) with 33% in a zygotene-like stage in which there appeared to be a block in pairing. Other cells (64%) reached pachytene, but with a greatly reduced frequency of recombination (mean 35, range 1-61); 52% of cells had unpaired regions, and 65% of cells had at least one bivalent with no recombination. The sex body was not formed in most cells. These studies in infertile men demonstrate a number of abnormalities in chromosome pairing and recombination associated with meiotic arrest.

**Prenatal level II mosaic marker representing true fetal mosaicism: What is the lesson?** *M. Nowaczyk, J. Xu.*  
Pathology & Molecular Medicine, Lab Medicine, Hamilton Health Sciences and McMaster University, Hamilton, ON, Canada.

We reported a case of a level II mosaic marker detected in amniocentesis that turned out to be a true fetal mosaicism. A 40-year-old woman had amniocentesis at 15.3 weeks gestation because of advanced maternal age. Routine G-banding analysis showed that 22 of the 23 in situ colonies had a normal male karyotype 46,XY. One colony had an extra small ring-like marker in 6/7 cells examined in that colony. A single abnormal colony is defined as level II mosaicism and is generally considered as pseudomosaicism, without significant clinical implications. Therefore, this finding was interpreted as a probable normal male karyotype; however, the possibility of fetal mosaicism could not be ruled out. A detailed fetal sonogram at 19 weeks gestation showed adequate fetal growth and no fetal anomalies. A normal male infant was born at term; there was a nuchal skin fold and a shawl scrotum. At 17 months of age he presented with a history of normal growth and normal gross and fine motor developmental milestones, however he had no intelligible speech. He never cooed or babbled as an infant. His hearing and understanding were normal; he would follow two-step commands. The follow-up cytogenetic analysis of cultured cord blood cells at birth showed a mosaic male karyotype with a marker in 14 (20%) of the 70 cells, 47,XY,+mar[14]/46,XY[56]. Both parents had a normal karyotype; thus it was a de novo marker. FISH revealed that the marker was negative for chromosome 15 probes specific for D15Z1 at 15q11.2, SNRPN at 15q11-q13 and PML at 15q22, and whole chromosome paint (wcp) 22. Spectral karyotyping using all 24 wcps did not produce a conclusive result likely due to the small size and makeup of the marker and/or a low proportion of the positive cells. This case suggests that while a sense of perspective should be kept, there exists a possibility of fetal mosaicism associated with a level II mosaicism found at the time of amniocentesis. Cytogenetic follow-up should be considered in such a case. Further case reports are needed for a better estimation of risks associated with level II mosaicism and for appropriate genetic counselling.

Program Nr: 786 from 2003 ASHG Annual Meeting

**Cytogenetic study of human lymphocytes under chloroquine treatment in vitro.** *C.V. Lopes, J.G. Frydman, I.P. Aranha.* Dept. de Bio Cel. e Genet., Univ. Est. do Rio de Janeiro, Rio de Janeiro, RJ , Brasil.

Chloroquine is a drug that was initially used for the treatment of malaria but that has also been successfully used for the treatment of arthritis and lupus eritematosus. The present study had the objective of studying the effect of chloroquine on chromosomes from human lymphocytes in vitro. Blood was collected from healthy donors 18 to 30 years old. Peripheral whole blood cells were incubated at 37C for 72h in enriched RPMI 1640 medium in the presence of chloroquine in the concentrations of 15, 30, 60 e 120 g/ml. Cells not exposed to the drug served as control for the experiment. A large amount of metaphases were obtained with the use of colchicine ( 16g/ml ). After fixation, chromosomes were stained with Giemsa Gurr ( 2% ) and analysed under optical microscope. Due to the low number of cells obtained in the 30 and 60g/ml concentrations and the lack of cells in the 120g/ml concentration, the groups were combined as exposed and not exposed to the drug. The low growth rate indicates that the drug had a cytotoxic effect. In 1416 metaphases analysed in the test group there were 704 with chromosomal alterations ( 1215 gaps and 545 breaks ). In the 1223 metaphases from the control group there were 24 abnormal ones ( 26gaps and 2 breaks ). The results were extremely significant (  $\chi^2$  with Yates correction = 746.78 ;  $p < 0.0001$  ) suggesting that chloroquine was responsible for the chromosomal alterations observed.

**Tertiary structure of cloned t(11;22) breakpoint DNA.** *H. Kurahashi*<sup>1,2,3</sup>, *B.S. Emanuel*<sup>2</sup>, *T. Toda*<sup>1</sup>. 1) Div Functional Genomics, Osaka Univ Grad Sch Med, Osaka, Japan; 2) Div Human Genetics, Children's Hosp Philadelphia, Philadelphia, PA; 3) Div Molecular Genetics, Inst Compre Med Sci, Fujita Health Univ, Aichi, Japan.

The constitutional t(11;22)(q23;q11) is the only known recurrent non-Robertsonian translocation. We have previously demonstrated that the translocation breakpoints are located within palindromic AT-rich repeats (PATRR) located on chromosomes 11q23 and 22q11. As a mechanism for this translocation, we proposed that the PATRR forms a cruciform structure that induces genomic instability leading to the translocation. To test this hypothesis, we have analyzed the tertiary structure of the PATRR cloned from 11q23. A plasmid containing the 11q23 PATRR undergoes a mobility shift upon agarose gel electrophoresis in a temperature-dependent manner. Analysis using two-dimensional gel electrophoresis indicates that the mobility shift is due to the formation of a cruciform structure. S1 nuclease or T7 endonuclease can cut the PATRR-containing plasmid into a linear form, which also suggests the cruciform formation. To visualize the tertiary structure of the PATRR, atomic force microscopy has been applied. Cruciform extrusions from the plasmid DNA with hairpin arms of the expected size were visualized. These data imply that the susceptibility to translocation mediated by the PATRR is likely a result of its unstable tertiary DNA structure.

**Phenotypic effects and pregnancy outcome of an unbalanced familial 5;21 chromosome translocation. C.**

*McKenna<sup>1</sup>, L.S. Heckman<sup>1</sup>, R. Schmidt<sup>1</sup>, M. Moretti<sup>2</sup>, A. Yenamandra<sup>3</sup>, P. Koduru<sup>3</sup>, L. Mehta<sup>3</sup>.* 1) Genzyme Genetics, Yonkers, NY; 2) St. Vincent's Medical Center, Staten Island, NY; 3) Schneider Children's Hospital & North Shore University Hospital, Manhasset, NY.

We describe a family where three members were found to carry an unbalanced 5;21 chromosome translocation. A 29 y.o woman was referred for amniocentesis because of increased nuchal skin fold in the fetus. Fetal karyotype was designated 46,XX,der(5)t(5;21)(q35;q22), with partial trisomy for 21q, including the Down syndrome critical region. Peripheral blood chromosome analysis of the pt. revealed a karyotype of 45,XX,der(5)t(5;21)(q35;q22),-21. FISH analysis confirmed the karyotypic findings. The pt.'s brother was independently evaluated for learning disabilities with multiple psychiatric diagnoses. He had a history of malignant hyperthermia during orchidopexy for undescended testes. He was short and had bilateral temporomandibular joint (TMJ) ankylosis. He carried the same translocation as his sister. The translocation was present in their mother. All three individuals had a similar appearance with prominent nasal tip, large ears and short stature. The sister also had TMJ problems, malignant hyperthermia and learning disabilities, but the mother denied these problems. The proband delivered a full-term female infant with features of trisomy 21. Cardiac findings were persistent left superior vena cava and patent foramen ovale. Other features present in the mother were not detected in the infant. The child died at 2 years following a respiratory illness. The familial translocation in our family was unbalanced with monosomies for distal 5q35-ter and proximal 21q. Adjacent meiotic segregation and fertilization with a normal sperm resulted in the infant's partial trisomy 21. The phenotypic features of distal 5q deletions and monosomy 21q22 are not well defined. TMJ instability and malignant hyperthermia have not been reported in association with these deletions and genetic susceptibilities for these problems have not been mapped to either region. A subtle though variable phenotype was associated with the der(5) chromosome in our patients, but could not be correlated with reports in literature.

**Telomere abnormalities with variable presentation: cytogenetic variants or a window to epigenetics?** *J.J.D Morrissette, L. Medne, J. Ganesh, K. Russell, DM. McDonald McGinn, EC. Moore, EH. Zackai, NB. Spinner.* Div Human Genetics, Children's Hosp Philadelphia, Philadelphia, PA.

Subtelomeric chromosomal rearrangements are emerging as an important cause of human malformations and neurodevelopmental disabilities. We have screened 1,297 patients using a subtelomeric probe set, and have identified 41 abnormalities that appear to correlate with clinical abnormalities (3.1%). In addition, we have identified 4 patients with a subtelomeric abnormality that was also found in one of their parents, raising the possibility that the finding may be a variant. Pt 1 had a PDA, VSD, ASD, TE fistula, duodenal atresia. Subtelomeric studies indicated a duplication of the 14q subtelomere probe (der(14)t(14;14)(p12;q32) which was found to be paternally inherited. There was no history of any medical problems in this patient's father. Pt 2 presented with clinical features of Goldenhar syndrome and subtelomeric testing revealed a deletion of 21q (46,XX,ishdel21q subtel(21qtel07-). Parental studies demonstrated that this deletion was maternally inherited. Pts 3 and 4 demonstrated the same subtelomeric deletion. The third patient presented with TOF, dysmorphic features and was found to have a deletion of 4q35 (46,XY, del(4)(q35).ishdel(4)(qtel)(4qtel11-). This deletion was visible as a subtle change on G-banding studies. Parental chromosomes demonstrated that the deletion was maternally inherited. The patient's mother demonstrated duplicated distal phalanges of the second toe and duplicated distal phalanges of the third toe on one foot, and small distal phalanges on the other foot, hearing loss, and learning differences with no identifiable syndrome. Pt 4 presented with IUGR, hypospadias and mild dysmorphic features. The deletion was not visible by G-banding, but subtelomeric studies demonstrated a deletion of 4q35, inherited from his healthy father. The significance of these subtelomeric abnormalities is not clear, but documentation of the frequency and clinical features associated with these abnormalities is needed. These may be normal variants, or they may represent abnormalities that show variable expressivity (possibly dependent on the genes associated with the other allele).

**Delineation of the clinical manifestations associated with an unbalanced familial chromosome abnormality:**

**46,XX,der(9)ins(9;11)(p23;??).** *J. Murphy*<sup>1</sup>, *E. Kolomietz*<sup>2</sup>, *I.E. Teshima*<sup>3</sup>, *D. Chitayat*<sup>1</sup>. 1) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto; 2) Department of Laboratory Medicine and Pathology, Mount Sinai Hospital, Toronto; 3) Department of Pediatric Laboratory Medicine, Hospital for Sick Children, Toronto.

Determining the clinical manifestations associated with abnormal karyotypes is an important tool for the identification of genetic disorders and the genes causing them. We present a family with microcephaly, mild to moderate mental retardation and mood disorder associated with duplication of a segment derived from chromosome 11. The proband was a 16-year-old girl in her second trimester of pregnancy who presented with a known 46,XX,9p+ karyotype, which was detected at the age of five when she, her sister and her mother were investigated for learning difficulties. Detailed assessment of the family members indicated that the proband and her mother have mild mental retardation, and her sibling has moderate mental retardation. All three have microcephaly and mood disorders, with the youngest age of presentation of depression being in the proband at age fourteen. A maternal aunt is described as having severe mental retardation and the maternal grandparents are described as having no learning difficulties. Chromosome analysis has not been performed to date on these individuals. Cytogenetic analysis done on the proband revealed a partial trisomy of chromosome 11 due to an unbalanced maternally inherited insertion: 46,XX,der(9)ins(9;11)(p23;??)mat. The insertion of chromosome 11 material into the chromosome 9 short arm was identified by SKY and confirmed by FISH using whole chromosome paint probes WCP9 and WCP11 (VYSIS) and a 9ptel FISH probe specific for the telomeric region of the short arm of chromosome 9 (Cytocell). Further molecular and cytogenetic investigations are underway to help define this region. This relatively large duplication of a segment from chromosome 11 is associated with a surprisingly little physical phenotypic effect in this family. The finding of the same mood disorder in all affected members of the family is important in determining genes associated with mood disorders.

**Selection of patients with idiopathic mental retardation for subtelomeric FISH analysis: Experience at Hospital for Sick Children, Toronto.** *R.E. Mueller<sup>1</sup>, G. Nie<sup>1</sup>, M.M. Nezarati<sup>3</sup>, L. Dupuis<sup>3</sup>, A. Teebi<sup>3</sup>, S. Kennedy<sup>3</sup>, D. Chitayat<sup>3</sup>, D. Stephens<sup>2</sup>, M. Shago<sup>1</sup>, I. Teshima<sup>1</sup>. 1) Cytogenetics, Department of Pediatric Laboratory Medicine; 2) Department of Population Health Sciences; 3) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, Canada.*

**Background:** Subtle chromosomal rearrangements are a common cause of mental retardation (Knight et al 1999). DeVries et al (2001) have proposed a five item checklist to improve the diagnostic pick-up rate among mentally retarded patients. After one year of experience using the Vysis ToTelVysion Multi-colour FISH Probe Panel at the Hospital for Sick Children, a large, tertiary care centre, we reviewed our results of testing for subtelomeric FISH rearrangement. **Design:** Clinical geneticists at the Hospital for Sick Children selected patients with idiopathic mental retardation and multiple congenital abnormalities suggestive of a chromosomal abnormality using their own criteria and subsequently evaluated the same patients using the deVries et al checklist. No particular score was required for testing. **Results:** Of 95 patients who had apparently normal karyotypes, seven patients were identified with subtelomeric chromosomal rearrangements: del(1)(p36p36)(CEB108/T7-), der(1)t(1;9)(p36.3;p24)(CEB108/T7+,305J7/T7+), del(6)(6PTEL48-), del(10)(D10S2490-), der(12)t(9;12)(q34.3;p13.1)(D9S325+,8M16/SP6-), der(12)t(12;16)(p13.3;p13.3)(8M16/SP6-,D163399+)pat, del(22)(q13.3)(ARSA-,D22S1726-). The detection rate was 7/95 or 7.4% (95% CI=2.1-12.6%). Two abnormalities were later judged to be familial variants when they were also identified in unaffected family members. The patients with normal subtelomeric regions had a mean score on the deVries et al checklist of 5.16 (SD 1.80, n=88) while those with subtelomeric changes had a mean score of 5.29 (SD 2.29, n=7). No one category made a disproportionate contribution to the total scores. **Conclusions:** Our patient selection is appropriate based on our pick-up rate. However, in agreement with deVries et al, we found the phenotype associated with subtelomeric chromosome changes is variable.

**Examination of the origin and mechanism of the idic(X) chromosome in case of Turner Syndrome with a mosaic karyotype.** *N.B. Kardon<sup>1</sup>, J.B. Ravnán<sup>2</sup>, R. Rapaport<sup>3</sup>, D. Bowlby<sup>3</sup>, B. Levy<sup>1</sup>, L. Edelmann<sup>1</sup>.* 1) Dept Human Genetics, Mount Sinai Sch of Medicine, New York, NY; 2) Genzyme Genetics, Santa Fe, NM; 3) Division of Pediatric Endocrinology, Mount Sinai Sch of Medicine, New York, NY.

The i(Xq) chromosome is the most common isochromosome and is present in 30% of all Turner syndrome females. In these cases the genetic defect can be viewed as both a sex chromosome aneuploidy and a chromosomal rearrangement disorder. We present the cytogenetic and molecular analysis of a case of classic Turner syndrome in a young woman with the mosaic karyotype: 46,X,idic(X)(p11.2)[13]/46,X,+mar[8]/46,X,+2mar[1]. Although the initial chromosome analysis of this patient, performed in The Republic of Georgia, was reported as 46,XX, our analysis of more than 150 cells failed to identify a single cell with two normal X chromosomes. The marker chromosomes were positive for X centromere material by Fluorescence In Situ Hybridization (FISH) and the presence of 2 centromeres was confirmed on the idic(X) using the DXZ1 probe (Vysis). Microsatellite analysis revealed that the idic(X) resulted from an intra-chromosomal exchange and was paternal in origin. Several BACs spanning 6 Mb on Xp11 were used as probes to narrow the breakpoints on Xp11. We determined that the breakpoints on Xp were proximal to the BAC RP11 348F1 on Xp11.22 at position 49.8 Mb on genomic contig NT\_011638. In addition, there was no evidence of euchromatic material on the marker chromosomes. Interestingly, FISH analysis performed with BAC RP11 38O23, which maps to the SSX (Synovial Sarcoma X) gene cluster, containing SSX5 and SSX6 on Xp11.22, also hybridized to a second locus more proximal on the normal X chromosome. This BAC had two positive signals on the idic(X) chromosome, however, the signal corresponded to the second more proximal locus, most likely the region containing the SSX2, SSX7 and SSX8 genes. The SSX genes on Xp11 are involved in the reciprocal somatic t(X;18)(p11;q11) that is characteristic of synovial sarcomas and generates a fusion gene between an SSX gene and the SYT gene on chromosome 18. We are currently investigating whether this repeated gene cluster was involved in the generation of the idic(X) in this patient.

**A rare cytogenetic compliment with two abnormal cell lines in a new born female.** *C.N. Harris<sup>1</sup>, A. Babu<sup>2</sup>, S. Kleyman<sup>2</sup>, M.J. Macera<sup>2</sup>.* 1) Long Island College Hospital, Brooklyn, NY; 2) Division of Molecular Medicine and Genetics, Department of Medicine, Wyckoff Heights Medical Center, Brooklyn, NY.

A 3555 gm baby girl was delivered by caesarian section at 39 weeks gestation of a 37 y. o. mother who refused amniocentesis. Remarkable features included low set ears, a large protruding tongue, symmetrical wide set nipples, shortened limbs, broad hands and a grade 1 to 2 systolic heart murmur. Chromosome analysis on peripheral blood lymphocytes, revealed two different abnormal cell lines. About 63% of her cells had a 45,X karyotype, while the remaining 37% cells had 46,XX,der(21;21)(q10;q10),+21. No normal cells were seen in 150 cells. Cytogenetic analysis of the father and a sibling showed normal karyotypes. Maternal specimen was not available for analysis. Considering the odds, based on the findings on the sibling and the family history, the mother is not likely to be a carrier of the der(21;21)(q10;q10). The occurrence of two unrelated abnormal cell lines, with no normal cells is very rare. There are only two previous reports of such cases with two abnormal cell lines, one with two aneusomic cell lines and the other the same as that seen in the present case. It is noteworthy that among such cases, two of these have a sex chromosome aneusomy coincident with an isochromosome 21. The most interesting is the mechanisms(s) giving rise to such a mosaic/chimera. The mechanism resulting in two aneusomic cell lines is obviously simpler than that required to produce an aneusomic cell line and a cell line with a structural rearrangement. One of the possible origins of such a chimera is a pre-zygotic event where two independent zygotes result from oocytes and/or a polar body, conjoined at early embryonic development. Alternatively, a series of post-zygotic events with concurrent loss of the normal cell line can explain the mosaic. Additional studies on DNA markers should enlighten the true mechanism involved in the present case.

**22q13.3 deletion syndrome : cryptic terminal rearrangements involving 22q13.3 is a significant cause of unspecified developmental delay and unexplained mental retardation.** *S.K. Murthy<sup>1</sup>, E. Lemyre<sup>2</sup>.* 1) Department of Pathology, Cytogenetic Laboratory, Hospital Sainte Justine, Montreal, PQ, Canada; 2) Departments of Pediatric and Medical Genetics, Hospital Sainte Justine, Montreal, PQ, Canada.

22q13.3 deletion, leading to a well recognized syndrome, is a subtle chromosomal change that may remain undetected by routine cytogenetic analysis. We report here a male child with global developmental delay, hypotonia, severe expressive language delay and minor dysmorphic features. Cytogenetic analysis showed a 47,XYY,rec(22)dup(22p)inv(22)(p11q13.2)mat.ish inv del(22)(TUPLE+, ARSA-,qtel-) karyotype. The abnormal chromosome 22 was inherited from his mother. She showed the presence of a pericentric inversion of chromosome 22 with no loss of the ARSA locus. The patient's normal younger brother carried the non recombined maternal inversion 22. One of the patient's maternal aunts with mental retardation also showed the same recombinant chromosome 22 with the 22q13.3 deletion. These clinical abnormalities in the patient and his aunt can be attributed to haploinsufficiency of genes in the deleted region of 22q13.3. Also to our knowledge, this is the first report of a recombinant 22 with 22q13.3 deletion in association with a sex chromosomal aneuploidy of XYY. The second case is a 10-month-old female with global developmental delay carrying a cryptic 22q13.3 deletion: 46,XX.ish del(22)(q13.3)(TUPLE+,ARSA-,qtel-). Her father showed an apparently balanced translocation between chromosomes 12 and 22 and the karyotype was interpreted as 46,XY.ish t(12;22)(q24;q13.3)(tel12q-,ARSA+,tel22q+;TUPLE+,ARSA-,tel22q-, tel12q+). FISH with 12q subtelomeric probe confirmed the paternal origin of the der(22) and trisomy for 12q subtelomere in the patient. The subtle nature of this chromosome change makes it difficult to detect the 22q13.3 microdeletion. Due to a non specific phenotype, this syndrome may be underdiagnosed clinically. Rigorous clinical assessment followed by high resolution chromosome analysis and FISH should be carried out to confirm this diagnosis, particularly in hypotonic children in whom other etiologies have been excluded especially in the presence of a major language delay.

**Developmental Genome Anatomy Project (DGAP): Identification of genes involved in human development.** *N.T. Leach*<sup>1,5</sup>, *G.A.P. Bruns*<sup>3,5</sup>, *R. Eisenman*<sup>3</sup>, *H.L. Ferguson*<sup>1</sup>, *J.F. Gusella*<sup>2,5</sup>, *D.J. Harris*<sup>1,3</sup>, *S.R. Herrick*<sup>1</sup>, *A.W. Higgins*<sup>1,5</sup>, *H.G. Kim*<sup>2,5</sup>, *A.H. Ligon*<sup>1,5</sup>, *W. Lu*<sup>1,5</sup>, *R.L. Maas*<sup>1,5</sup>, *S. Michaud*<sup>1,5</sup>, *A.M. Michelson*<sup>1,4,5</sup>, *S.D. Moore*<sup>1,5</sup>, *R.E. Peters*<sup>1</sup>, *B.J. Quade*<sup>1,5</sup>, *F. Quintero-Rivera*<sup>2,5</sup>, *R.E. Williamson*<sup>5</sup>, *C.C. Morton*<sup>1,5</sup>. 1) Brigham & Women's Hospital, Boston, MA; 2) Massachusetts General Hospital, Charlestown, MA; 3) Children's Hospital, Boston, MA; 4) Howard Hughes Medical Institute; 5) Harvard Medical School, Boston, MA.

The Developmental Genome Anatomy Project (DGAP, <http://dgap.harvard.edu>) is a collaborative effort to identify genes important for human development. Breakpoints of balanced chromosomal rearrangements in individuals with congenital anomalies are analyzed in an attempt to uncover potential causal relationships between rearrangements and phenotypes. A benefit to the individual and geneticist alike is a possible explanation of a disorder that may be otherwise undefined. Our study takes a high-throughput approach by utilizing four groups expert in: (1) patient identification and sample collection, (2) FISH-based breakpoint localization, (3) breakpoint cloning and candidate gene identification, and (4) functional analysis in model organisms. More than 126 cases have been ascertained through collaborations with clinicians, cytogeneticists and genetic counselors. To date, 62 breakpoints have been FISH-mapped, 27 of which have been positioned on the human genome map within a single BAC clone. Fifteen breakpoints have been localized further to 0.5-36 kb, and 8 have been cloned. In 8 cases the breakpoints have been shown to disrupt sequences of known genes. Surprisingly, the presence of a microdeletion at the breakpoint was found in only 1/27 cases. Attractive candidate genes such as *DGKD* for a seizure phenotype, *MTAP* and *FLJ21820* for hearing loss, *FGFR1* for Kallman syndrome and several others, are under extensive molecular study. Knock-out mouse models for *FLJ21820* and *DGKD* orthologs are being created for functional testing. DGAP success in gene discovery is built on the selfless dedication of the medical genetics community and will continue to provide crucial insights into human development.

**Molecular Evaluation of Maternal Cell Contamination in Spontaneous Abortions Cultured Cells.** *S. Nazarenko, T. Nikitina, I. Lebedev, N. Sukhanova, E. Sazhenova, M. Nazarenko, A. Beljaeva.* Cytogenetics Lab, Inst Med Gen, Tomsk Science Ctr, Tomsk, Tomsk, Russia.

Estimation of the chromosome constitution of the fetus by long-term tissue cell cultures is prone to error due to contamination of such cultures by maternal cells. Only a few studies have been performed to discriminate between true confined placental mosaicism and maternal cell contamination (MCC) by molecular methods. In the present study, we examined the results of retrospective cytogenetic analysis of 478 first trimester spontaneous abortions. In 16% of the embryos with a 46,XX karyotype a molecular assay of the amelogenin locus revealed the presence of Y-chromosome DNA in non-cultured embryonic tissues. There was a significantly prolonged cell cultivation time prior to chromosome harvesting for these conceptions in comparison with abortions without a Y-chromosome. A mathematical model for estimation of MCC level, expected rates of different karyotypes in abortions with correction for MCC and diagnostic accuracy of conventional cytogenetic analysis is proposed. Taking MCC into account, the frequency of chromosomal abnormalities in the studied sample of spontaneous abortions rose from 54.6% to 60.3% and the sex ratio in abortions with normal karyotype increased from 0.66 to 1.02. The model allows to evaluate the diagnostic accuracy of cytogenetic analysis, which make up to 92.3%. The experimental validation of the proposed model was performed using several highly polymorphic microsatellite DNA markers for chromosomes 2, 11, 16, 19, 20 and 21 in 60 46,XX embryos and their parents. For 7 abortions (11.7%), including 4 without molecular evidence for a Y-chromosome, numerical chromosomal abnormalities were revealed and confirmed by FISH. This frequency corresponds well to the expected rate 8.3%, predicted by the model and study design. MCC clearly affects the rates of chromosomal abnormalities and sex ratios in spontaneous abortions. Thus, correction for MCC should be taken into account before invoking biological explanations of sex ratio bias, such as the effect of X-linked lethal genes or abnormal X-inactivation, and may be useful to include in diagnostic reporting.

**Meiotic 3:1 segregation outcome in POCs and livebirths.** *M. LeMieux, K. Reddy.* Genzyme Genetics, Orange, CA.

Our aim is to assess the empirical distribution of 3:1 segregants of balanced reciprocal translocations and the clinical impact. In 40 cases compatible with a 3:1 segregation, 15 were products of conception (POC) and 25 were livebirths. Parental chromosome(cs) studies showed maternal inheritance in 21(81%), paternal in 4(15%) and de novo in 1(4%) case. In POC and livebirths the 3:1 segregation outcomes were: tertiary trisomy (TT) with a supernumerary derivative cs in 60% [9/15] and 64% [16/25], interchange trisomy (IT) for a whole chromosome due to two translocation cs and a normal homolog segregation in 7% [1/15] and 28% [7/25], and tertiary monosomy (TM) for one of the derivative cs in 33% [5/15] and 8% [2/25]. The TT with supernumerary der(22)t(11;22), was seen in 5 cases. An acrocentric cs was involved in all livebirth cases and 47 % of POCs. The TT POCs had a derivative cs that was on average (2.2 fold) larger than in livebirths. IT usually involved non-viable trisomies (3, 10, 15, 9 & 21) in POC as compared to livebirths (21). TM had a loss of an acrocentric cs 22(50%), 21(25%), or 15(25%), which was involved in the translocation. The other derivative cs present was usually a nonacrocentric and in 7/8 cases the breakpoint was in the terminal band. Hence, the loss was minimal and the gain was partial long arm of 21, 22 or 15. In many cases FISH studies were necessary to characterize or confirm the origin of the derivative cs. The clinical spectrum ranged from mild to severe. The cases with apparently normal to mild phenotype were TT and TM found in mothers with an abnormal amniocentesis result. A TT found in a 37-year-old fertile woman whose mother carried a balanced translocation. Two siblings, one a 35-year old apparently normal female with TT and the other 37-year-old with TM resulting from 3:1 segregation of maternal t(6;21) (q27;q21.2). A 39-year-old patient with premature menopause and TM, 45,XX,der(18)t(18;22) (p11.3;q11.2),-22.ish der(18)(ARSA+,TUPLE+,D14Z1/D22Z1-,D18S552-,D18Z1+,MBP+) karyotype. All other cases had congenital abnormalities. Included in this group, was a newborn with TM, 45,XY, der(13)t(13;15)(p11.2;q15),-15 pat.ish der(13) (GABRB3-,PML+,D13Z1/D21Z1+) karyotype and Prader-Willi syndrome.

Program Nr: 798 from 2003 ASHG Annual Meeting

**The double minutes chromosomes in workers exposed to densely-ionising (high-LET) radiation.** *N. Popova, V. Puzyrev, S. Nazarenko.* Institute of Medical Genetics, Tomsk, Russia.

The analysis of peripheral blood lymphocytes was performed in the 60 healthy workers of Nuclear-Chemical Plant (NCP) who have various internal Pu-239 burdens (I group - 1,5-11 nCi; II group - 13-25 nCi and III group - more 40 nCi). As control 36 unexposed persons from ecologically clean village were observed. In average 300 cells per individual were analyzed. Double minutes chromosomes (DMs) have been found only in plutonium workers of II and III groups. In II group 10 from 22 studied individuals and in III group 7 from 24 persons had cells with DMs. It was found 23 (II group) and 10 (III group) cells with DMs per 5778 and 6560 metaphases (0.350.10 and 0.150.05 percent) respectively. Differences between II and III groups of plutonium workers and control individuals were statistically significant ( $P < 0.01$ ). We believe, that DMs in peripheral blood lymphocytes of plutonium workers may be a biomarker of densely-ionising internal irradiation and can display an initial stage of cell transformation. Plutonium workers who have cells with DMs should be periodically to undergo cytogenetic testing and frequent clinical monitoring for early detection of possible oncological pathology.

**Novel X:Y translocation in a female habitus with dysmorphic features and implications for sex reversal. A.**

*Maleki*<sup>1,2,3</sup>, *D. Manchester*<sup>1,3</sup>, *C. Donovan*<sup>2</sup>, *N. Parker*<sup>2</sup>, *J. Kennaugh*<sup>4</sup>, *L. McGavran*<sup>1,2,3</sup>. 1) University of Colorado Health Sciences Center-Department of Pathology; 2) Colorado Genetics Laboratory; 3) The Children's Hospital-Department of Pediatrics; 4) Presbyterian/St. Luke's Hospital, Denver, CO.

We report the case of a novel X;Y translocation female habitus newborn referred for standard cytogenetic testing and a FISH trisomy panel to rule out an abnormality consistent with Trisomy 18 features. Although preliminary FISH findings were negative for trisomy 13, 18 and 21, she had the presence of a Y chromosome [Ycen (DYZ3)]. Clinical findings at birth included intrauterine growth retardation, agenesis of the corpus callosum, a Dandy Walker variant, telecanthus, mild retrognathia, overlapping fingers, an atrial septal defect, seizures, incomplete formation of the labia majora superiorly, and a prominent clitoris. The distal vagina appeared normal. A well formed uterus was seen on ultrasound and renal ultrasound was normal. Standard cytogenetic analysis revealed a 46,X,der(Y)t(X;Y)(p11.23;q11.222) karyotype as the sole abnormality found in all cells examined. To further characterize the der(Y), additional FISH studies were performed which confirmed the presence of the SRY locus, absence of sat III Yqh, presence of both Xp and Yp subtelomeric sequences, presence of the Kallman locus, and normal 9ps. This confirmed the presence of an abnormal sex chromosome including the p arms of both the X and Y chromosomes. There are few cases of female der(Y)t(X;Y) in the literature. Bardoni et al.,(1993) describe a dosage sensitive sex reversal gene (DSS) on Xp21 which, when duplicated, or present without an XIST gene, can result in a phenotypic female, even in the presence of SRY. The case presented here results in a novel der(Y), including Y short arm with minimal Yq, and whole X short arm with the lack of an Xq, which results in functional disomy of the majority of the genes present on Xp, and three copies of the pseudoautosomal region Xp/Yp. The more involved phenotype of this child is most likely due to deletion of the X-inactivation center and the resultant lack of dosage compensation of the genes present on Xp.

**Analysis of a translocation breakpoint by FISH and PRINS: a molecular cytogenetic approach.** *M. Nimmakayalu<sup>1</sup>, A.L. Gotter<sup>1</sup>, B.S. Emanuel<sup>1,2</sup>*. 1) Human Genetics & Molecular Biology, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Pediatrics, University of Pennsylvania. School of Medicine, Philadelphia, PA.

Low copy repeats (LCRs) located in 22q11.2, especially LCR-B, are susceptible to rearrangements associated with several common constitutional disorders. These include DiGeorge syndrome, Velocardiofacial syndrome, and recurrent translocations of 22q11 including the constitutional t(11;22) and t(17;22). The presence of palindromic AT rich repeats (PATRRs) within LCR-B of 22q11.2, as well as within the 11q23 and 17q11 regions, has suggested a palindrome-mediated, stem loop mechanism for the generation of recurrent constitutional 22q11 translocations. Several additional autosomes and the X chromosome, are involved in non-recurrent rearrangements with the PATRR of LCR-B of 22q11.2. We have adapted a molecular cytogenetic approach in the form of fluorescence in-situ hybridization (FISH) and Primed in-situ hybridization (PRINS) to rapidly localize the BP of one of these sporadic 22q11.2 translocations, a t(4;22). This is the first time PRINS has been used to fine-map a translocation breakpoint (BP). Our approach localized the t(4;22) BPs within 4q36 and 22q11.2, and permitted cloning and analysis of translocation junction fragments. By FISH, a 200 kb BAC clone crossing the translocation BP was identified on chromosome 4. Multiple primer pairs were designed from the sequence of the BP-spanning BAC to use for PRINS reactions. Amplification of adjacent primer pairs, labeled in two colors, allowed us to narrow the t(4;22) BP to a 6.7 kb clonable region. Identical primer pairs were used for PCR mapping of t(4;22) somatic cell hybrid DNA, confirming the PRINS localization results. The PRINS approach allowed rapid BP cloning and demonstrated the presence of a 270 bp palindromic sequence at the chromosome 4 BP. Analysis of this BP further supports a stem loop secondary structure mechanism for the formation of other, non-recurrent translocations with 22q11.

**Tandem duplication of Xq13->q22 in a newborn female with hydrocephalus.** *P. Mowrey<sup>1</sup>, L. Bason<sup>2</sup>, E. Arch<sup>2</sup>, I. Gadi<sup>1</sup>, L. Wisniewski<sup>1</sup>, J. Tepperberg<sup>1</sup>, P. Singh-Kahlon<sup>1</sup>, P. Papenhausen<sup>1</sup>.* 1) Diagnostic Genetics, Laboratory Corp of America, Res Triangle Pk, NC; 2) Division of Medical Genetics, A.I. DuPont Hospital for Children, Wilmington, DE.

A 6 month old female presented at birth with hydrocephalus and later with decreased muscle tone, DD and FTT. Shunting was performed at one month. Standard cytogenetics at 6 months revealed an extra band segment within one of the X chromosomes at Xq13. Chromosome paint confirmed an X derivation and high resolution analysis suggested direct tandem duplication of X(q13->q22). Interestingly, the abnormal X appeared to be active in most cells by virtue of scoring inactivation folding, suggesting partial functional disomy in a significant proportion of cells. Replication studies and parental karyotyping are in process. In general, short stature, hypotonia, and in some cases MR, are characteristic of Xq duplications in females. However, no previous case was apparently associated with hydrocephalus nor did any of these appear to involve the q13->q22 region. The abnormal X in previous studies in the literature has not always been inactive so that functional partial disomy was present. It is likely that functional nullosomy present in X deletions results in much faster and complete inactivation skewing due to strong selective disadvantage early in embryonic development which may account for the milder deletion phenotypes compared to duplications.

**De Novo 46,XX,del(4)(q12q13.3) in a 3 year-old with piebald trait.** *D. LaGrave<sup>1</sup>, M.S. Eswara<sup>2</sup>, S.P. Yang<sup>2</sup>, M. Tomlinson<sup>1</sup>, N. Qin<sup>1</sup>.* 1) Cytogenetics Department, Quest Diagnostics Nichols Institute, San Juan Capistrano, CA; 2) GenetiCare Medical Associates, Davis, CA.

We describe a 3 year-old girl born at term to a 24 year-old gravida 5, para 2, SAB 3, phenotypically normal mother. Amniocentesis was performed due to two previous pregnancies with neural tube defects that resulted in intrauterine fetal demise at 32 and 37 weeks, respectively. Chromosome studies on the amniocytes revealed an interstitial deletion in the long arm of one chromosome 4. However, a series of ultrasounds were negative for any adverse findings. At birth, the infant weighed 6 lb, 15 oz, was 20 inches in length, and had an OFC of 33cm. No neonatal problems were reported. Findings from a CT scan of the head and an echocardiogram were reportedly negative. Hearing and vision were normal at birth, although esotropia was diagnosed at 18 months. The patient shows delay in all milestones. She is estimated to be functioning at a 6-month level for speech and communication and at a one-year level socially. She cannot yet eat solid foods. Her weight is at the 97<sup>th</sup> percentile, her height is at the 75<sup>th</sup> percentile and her OFC is at the 50<sup>th</sup> percentile. She displays subtle dysmorphic features including a high forehead, hypertelorism, a short nose, and a small midface. Her ears are low-set and posteriorly rotated, but with normal morphology. Her teeth were late in erupting, are yellowish in color and seem to have abnormal enamel. She has a midline white forelock and several large hypopigmented areas on her trunk and lower extremities. A few smaller areas of hyperpigmentation are present as well. She has had no significant medical problems or surgeries. Repeat karyotype of peripheral blood lymphocytes was performed at 3 years of age. This confirmed the previous finding of an interstitial deletion on 4q and identified the breakpoints as 4q12q13.3. FISH analysis using a whole chromosome paint probe specific for chromosome 4 revealed that the missing segment from 4 was not present on any other chromosome. Parental chromosome studies were normal. In summary, we discuss a rare deletion on 4q that resulted in developmental delay, mild dysmorphic features, and piebald trait.

**Quantification of aneuploid fetal cells in peripheral blood of pregnant women using molecular cytogenetic techniques.** *K. Krabchi<sup>1</sup>, M. Gadjji<sup>1</sup>, L.G. Jackson<sup>3</sup>, M. Ferland<sup>1</sup>, S. Roy<sup>1</sup>, M. Bronsard<sup>1</sup>, J.C. Forest<sup>2</sup>, R. Drouin<sup>1</sup>.* 1) Human & Molecular Genetics Unit, Medical Biol, St Francois d'Assise Hosp, Laval Univ, Quebec, PQ, Canada; 2) Perinatal Unit, St Francois d'Assise Hosp, Laval Univ, Quebec, PQ, Canada; 3) Dept Obstetrics & Gynecology, Drexel Univ College of medicine, Philadelphia, PA, USA.

Background: Fetal nucleated cells circulating in the peripheral blood during pregnancy are potential targets for noninvasive genetic testing. Molecular cytogenetic techniques have provided definitive evidence of their presence. In a previous study, we have shown that it is possible to reliably and reproducibly identify fetal cells in maternal blood of all pregnant women and to quantify all fetal nucleated cells (Krabchi K. et al Clin Genet 60:145, 2001). This number fluctuated between 2 to 6 cells per mL of maternal blood between 18th and 22nd weeks of gestation in euploid pregnancies. Objective: The aim of our study is to determine, using the same approach, the total number of fetal nucleated cells in maternal blood of pregnant women bearing an aneuploid conceptus. Methods: In order to achieve maximum recovery of fetal cells, we used a simple and rapid harvesting method without any enrichment procedures, followed by either Fluorescent in situ hybridization (FISH) or Primed IN Situ (PRINS) labeling technique. Detection of fetal cells was carried out using chromosome-centromeric or locus specific probes directly coupled to fluorochromes for FISH (CEP-X a-sat, CEP-Y sat III, CEP-18 a-sat, LSI-21, Vysis Inc.) or specific primers for chromosomes X, Y, 18, 7 and 8, for PRINS reaction. PRINS or FISH techniques were performed on blood specimens provided by 39 healthy pregnant women carrying an aneuploid fetus: 47,XY,+21 (12 cases); 47,XX,+21 (9); 47,XY,+18 (6); 47,XX,+18 (2); 47,XY,+13 (1); 47,XXX (2); 47,XXY (2); 47,XYY(1); 45,X (1); 69,XXX (1) and 69,XXY (2). Results: Between 4 and 32 fetal cells per mL were detected. These numbers are on the average 5-fold higher than the number of fetal cells observed in euploid pregnancies. Conclusion: The number of fetal cells in maternal circulation is remarkably higher in aneuploid pregnancies.

**Loss of abnormal cell line in chromosomal mosaic syndromes is due to increased cell death.** *S.L. Wenger, W. Tang.*  
Pathology, West Virginia University, Morgantown, WV.

Chromosomal mosaic syndromes, such as i(12p), i(9p) and trisomy 8 mosaicism, are characterized by a decrease of the abnormal cell line with age of patient, in vivo, or passaging of cells, in vitro. It has been thought that this is due to selective growth advantage of the normal cell line. We have shown previously that the cell cycle kinetics is similar for normal and trisomy 8 cells within the same tissues from an individual with trisomy 8 mosaicism (Hulley BJ et al. *Am J Med Genet* 2003;116A:144-6). Cell cycle kinetics was performed on i(9p) peripheral blood cells, which showed a similar rate of cell division for the normal and abnormal cells. In addition, amniocytes from an i(12p) fetus were examined for cell death using annexin V stain. A normal diploid control and i(12p) cultured cells showed annexin V fluorescent staining in the cytoplasm of 9% and 7% of cells, respectively. Additional cells were fixed with formalin and processed for FISH using a probe for the centromere of chromosome 12. These same cells were then stained with annexin V. In the normal control, 29% amniocytes stained positive for annexin V, suggesting the fixative process affected the cell membrane, thereby increasing the annexin V staining. In the mosaic amniocytes, cells with 2 signals (normal) had 28% annexin V positive cells, similar to the control cells. However, cells with 3 signals (i(12p)) had 43% annexin V positive cells, which is 50% higher than normal diploid cells ( $p < 0.1$ ). Although not statistically significant due to fewer aneuploid cells than diploid, the results suggest that the abnormal cell line in chromosomal mosaic syndromes decrease over time due to a higher rate of cell death, and therefore a growth disadvantage of the abnormal cell line.

**Mosaic sex chromosome anomalies in a normal appearing male newborn: prenatal and postnatal molecular cytogenetics studies.** *J. Xu<sup>1,2</sup>, M.J. Huggins<sup>3</sup>, T. Heshka<sup>1,2</sup>, S.A. Farrell<sup>4</sup>, M.J.M. Nowaczyk<sup>1,2</sup>.* 1) Pathology and Molecular Medicine; 2) Lab Medicine; 3) Obstetrics and Gynecology, Hamilton Health Sciences and McMaster University; 4) Lab Medicine, Credit Valley Hosp, Mississauga, ON, Canada.

A 40-year-old mother at 15.1 weeks gestation had amniocentesis for advanced maternal age. G-banding showed 11 of 12 in situ colonies had a marker, 46,X,+mar and 1 colony had 45,X. FISH of 37 subculture cells from 6 colonies, using centromere X (CEPX) and Y (CEPY) probes, and SRY at Yp11.3 showed 4 cell lines and 2 types of marker, both being of Y origin. One marker had single CEPY (12 cells) or SRY signal (14 cells), which was interpreted as deletion Yq, del(Y)(q11). Another marker had double CEPY signals in the proximal region (2 cells) or double SRY signals, one at each end (1 cell). This was interpreted as isodicentric Y, idic(Y)(q11). Two of the 4 cell lines were male; 46,X,del(Y)(q11)(26/37, 70%) and 47,XX,idic(Y)(q11)(3/37; 8%), and 2 were female; 45,X (2/37, 5%) and 46,XX (6/37, 16%). Presence of Y material (especially SRY) in most of the 37 subculture cells and 12 colonies was consistent with the ultrasound finding of a male fetus with no evidence of malformations. The couple was informed that such a prenatal finding could be associated with a wide phenotypic range, with a phenotypically normal male baby being likely, and with a significant risk of a male being infertile. After counseling, the couple chose to continue the pregnancy. The male infant was born at term and was normal in appearance. At birth, G-banding of the cultured cord blood cells showed 3 cell lines: 46,XX[2/50,4%], 46,X,del(Y)(q11)[33/50, 66%], and 46,X with a small ring[15/50,30%]. FISH confirmed the presence of SRY or CEPY signals in the marker. G-banding of the cultured cord tissue cells also showed 3 cell lines: 46,X,del(Y)(q11)[37/50, 74%], 47, XX, idic(Y)(q11)[4/50, 8%], and 45,X[8/50,16%]. In addition, 1 cell was 46,X with a large ring. This case suggests that a prenatal finding of multiple sex chromosome anomalies involving del(Y)(q11), idic(Y)(q11), r(?Y), or 45,X might not have external phenotypic effects in a male baby when the majority of the cells have 46,X, del(Y)(q11) and intact SRY.

**The 10q telomere region is a common site mediating polymorphic and pathogenic telomere rearrangements. D.**

*Waggoner<sup>1</sup>, D. Eash<sup>1</sup>, Y. Ilkin<sup>1</sup>, U. Surti<sup>2</sup>, E. McPherson<sup>2</sup>, M. Siegler<sup>3</sup>, J. Tepperberg<sup>4</sup>, C. Lese-Martin<sup>1</sup>.* 1) Dept Human Genetics, The Univ Chicago, Chicago, IL; 2) Pathol Gen, Magee Women's Hosp, Univ Pittsburgh, Pittsburgh, PA; 3) Clinical Genetics Center, Univ of Wisconsin, Madison, WI; 4) Cytogenetics Lab, LabCorp, RTP, NC.

With the increased utilization of genome-wide telomere screening it has become evident that as many as 5% of patients with previously unexplained mental retardation and/or birth defects have subtle rearrangements involving the telomeres. During the course of these studies, telomere rearrangements have been identified in normal individuals, suggesting that not all rearrangements are associated with a clinical phenotype. For example, deletions of the 2q and X/Yp telomere regions are typically inherited from a normal parent and are well described common polymorphisms. We report 6 patients with either a partial deletion, complete deletion or duplication of the 10q telomere probe, BAC 261B16. Family studies revealed that three of the four deletion cases and both duplication cases were inherited from unaffected parents. The fourth deletion case, a partial deletion, was *de novo*. Extended family mapping studies of the two families with complete deletions of the 10q telomere probe revealed that the deletions were not the same size, one was 1.4 Mb and the other 1.5 Mb, and that these deletions do not appear to correlate with the phenotypic features found in the families. This data suggests that 10q telomere deletions or duplications are often inherited and most likely represent polymorphisms. In addition, we have also observed that the 10q telomere region seems to be a common site for more complex balanced and unbalanced rearrangements. The combined experience of the two labs show that of 113 abnormal telomere cases, 12 (10.6%) involved abnormalities of 10q (including partial deletions, full deletions, duplications, reciprocal chromosomes and unbalanced translocations). Taken together, these data suggest that the 10q telomere region is a common site mediating telomere rearrangements leading to both inherited polymorphisms and unbalanced rearrangements associated with clinical perturbations.

**Prenatal diagnosis of a complex de novo translocation leading to sex reversal in a fetus.** *G.V.N. Velagaleti<sup>1,2</sup>, J.K. Northup<sup>2</sup>, S. Surendran<sup>1</sup>, M.R. Grafe<sup>2</sup>, J.M. McKee<sup>3</sup>, L.H. Lockhart<sup>1</sup>, S.M. Jalal<sup>4</sup>.* 1) Departments of Pediatrics; 2) Pathology; 3) Family Medicine, University of Texas Medical Branch, Galveston, TX; 4) Department of Pathology, Mayo Clinic, Rochester, MN.

A 15-year-old, G1P0 Caucasian female was referred for chromosome analysis at 12 weeks gestation due to family history of Down syndrome. Ultrasound examination showed multiple abnormalities including, increased nuchal fold, prominent cisterna magna, micrognathia, deviation of cardiac axis and hypoplasia of middle phalanx of the fifth digit. Chromosome analysis from amniocytes showed a complex balanced translocation, 46,XY,t(4;7;8;17)(4qter->4p15.2::17q25->17qter;7qter->7p15::4p15.2->4pter;8pter->8q12.2::7p21.2->7pter;17pter->17q25::8q12.2->8qter). Additional FISH with painting, subtelomeric probes and M-FISH studies confirmed the complex nature of the translocation. The patient elected to continue the pregnancy. A baby girl was delivered at 38 weeks of gestation. The infant had several congenital anomalies including a cleft palate, micrognathia, small mouth, posteriorly rotated ears, nail and digital abnormalities. Due to severe respiratory complications the baby died after 3 weeks. The autopsy results indicated several skeletal anomalies including 11 pairs of short and wavy ribs with deficient cartilage anteriorly. Though the infant had normal female external genitalia, the ovaries under microscopic examination were devoid of oocytes. The congenital anomalies seen in this infant are consistent with acampomelic campomelic dysplasia (acampomelic CMPD). Acampomelic CMPD is known to be caused by haploinsufficiency of SOX9 gene mapped to chromosome 17q25 which is one of the breakpoints seen in our case. There have been reports of balanced chromosome translocations involving 17q25 leading to ACMD with sex reversal, but this is the first report of Acampomelic CMPD caused by a complex de novo translocation diagnosed prenatally. Additional studies to determine the involvement of SOX9 are in progress.

**Bivalent chiasma frequencies in individual chromosomes using cenM-FISH analysis of human pachytene cells. F.**

*Sun*<sup>1</sup>, *M. Oliver-Bonet*<sup>2</sup>, *T. Liehr*<sup>3</sup>, *K. Trpkov*<sup>4</sup>, *E. Ko*<sup>5</sup>, *J. Navarro*<sup>2</sup>, *J. Benet*<sup>2</sup>, *R.H. Martin*<sup>1,5</sup>. 1) Medical Genetics, University of Calgary, Calgary, AB, Canada; 2) Unitat de Biologia, Universitat Autònoma de Barcelona, Bellaterra, Spain; 3) Institute of Human Genetics and Anthropology, Jena, Germany; 4) Pathology, Rockyview Hospital, Calgary, AB, Canada; 5) Alberta Children's Hospital, Calgary, AB, Canada.

There is very little information on the number of chiasmata in individual human germ cells. Chiasmata in individual human chromosomes have been assessed from diakinesis preparations using conventional cytogenetics. However, diakinesis preparations are very difficult to analyze as chromosome morphology and banding are poor, making identification of individual chromosomes and chiasma sites challenging. Nevertheless, these studies on a small number of men are widely cited to give estimates on recombination frequencies of individual chromosomes. A new immunocytological approach allows analysis of pachytene cells by using antibodies to detect the synaptonemal complex (SCP1/SCP3), the centromere (CREST) and sites of recombination (MLH1). We have used this approach in combination with centromere-specific multicolour (cenM-)FISH to identify chiasma sites in individual chromosomes in normal men for the first time. The men had a mean of 48 recombination foci (range 34-62) in all autosomes. For individual chromosomes, the mean number of chiasmata for chromosomes 1 to 22, respectively, was 3.85 (1), 3.60 (2), 2.90 (3), 2.45 (4), 2.75 (5), 2.65 (6), 2.60 (7), 2.20 (8), 2.40 (9), 2.30 (10), 2.40 (11), 2.55 (12), 2.00 (13), 2.05 (14), 1.90 (15), 2.15 (16), 2.25 (17), 1.90 (18), 2.00 (19), 1.85 (20), 1.00 (21), and 1.15 (22). This method allows precise identification of the number and position of recombination foci in individual chromosomes.

**Cytogenetic Study of Human Lymphocytes Exposed to Methylparaoxon *in vitro*.** P.B. Souza, E.D. Sodr , I.P. Aranha. Dept. de Bio Cel e Genet., Univ. Est. do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brasil.

Methylparaoxon is an organophosphate pesticide largely used in agriculture. In order to exert its biological effect it must be biotransformed into its corresponding oxon-analogous, methylparaoxon, in the liver. The objective of the present work was to study the effect of methylparaoxon on chromosomes of human lymphocytes *in vitro*. Blood was collected from healthy donors 18 to 30 years old. Cells were exposed to the drug at the concentrations of 0.1, 0.25 and 0.5 ppm. Cells not exposed to the drug served as control for the experiment. Peripheral whole blood cells were incubated at 37 C for 72h in enriched RPMI 1640 medium. A larger number of metaphases were obtained with the use of colchicine (16 g/ml). Chromosomes were stained with Giemsa Gurr (2%) and analysed under optical microscope. At the 0.1 ppm concentration, in 549 metaphases, there were 343 normal ones and 206 with chromosomal abnormalities (264 gaps and 113 breaks). At the 0.25 ppm concentration 500 metaphases were analysed. Of those, 215 were normal and 285 abnormal (438 gaps and 133 breaks). At the 0.5 ppm concentration, 439 metaphases were observed. Of those, 205 were normal and 234 showed chromosomal abnormalities (353 gaps and 66 breaks). In the control group, in 500 metaphases studied, 472 were normal and 28 were abnormal (32 gaps and 7 breaks). The results obtained were extremely significant ( $\chi^2=343.5$ , p 0.0001). They suggest that methylparaoxon was responsible for the chromosomal abnormalities observed.

**Familial case of duplication 10p without phenotypic effects.** *D. Saxe*<sup>1</sup>, *K. Coleman*<sup>2</sup>, *D. Miley*<sup>1</sup>, *A. Yearall*<sup>1</sup>, *T. Sanders*<sup>1</sup>, *K. May*<sup>1</sup>. 1) Human Genetics, Emory Univ Sch of Medicine, Decatur, GA; 2) Children's Hospital of Atlanta, Atlanta, GA.

We describe a family with a history of congenital heart defects and an unrelated inherited duplication of the short arm of chromosome 10 (pterp13::p14qter). The first family member, a 19 yr. old female, was referred for amniocentesis due to low serum screen at 19.5 weeks gestation. Chromosome studies revealed extra material at 10p13. The mother's blood revealed the same abnormal 10 with no other chromosome abnormality. FISH with Coatosome 10 paint demonstrated the material to be chromosome 10 in origin. This pregnancy, and a second, with normal chromosomes, had normal outcomes. A third child had no prenatal diagnosis, but was born with tetralogy of Fallot. Cytogenetic studies at birth determined that this child carried the dup(10)(p13). FISH for DiGeorge syndrome (DGS) I and II on chromosomes 22 and 10 were negative. A family history revealing multiple relatives with either tetralogy of Fallot or other congenital heart defects prompted three other family members to have chromosome studies. These studies showed that the heart defects were unrelated to the abnormal 10p. Comparative genomic hybridization showed that the abnormal 10p is due to a duplication rather than an inversion. Therefore, this area of 10p appears to be a region which can be duplicated without phenotypic effect.

**Familial duplication, dup(11)(p14p15), in a mother and 9-year-old son confirmed by high resolution CGH.** *H.E. Wyandt*<sup>1,2</sup>, *X.L. Huang*<sup>1</sup>, *J.M. Milunsky*<sup>1,3,4</sup>. 1) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 2) Dept of Pathology; 3) Dept of Pediatrics; 4) Dept of Genetics and Genomics.

A 9-year-old African-American male has been followed since 2 years of age due to his mental retardation, severe behavioral problems and dysmorphism. Initial cytogenetic analysis and chromosome painting revealed an apparent duplication in the short arm of a chromosome 11, seen also in his mentally retarded mother. The proband has moderate to severe mental retardation, a history of IUGR, infantile hypotonia, FTT, exotropia, inguinal hernia repair, and several dysmorphic features. His mother has mild mental retardation, a history of impulsivity, assaultive outbursts, and similar dysmorphism.

Although G-banding and FISH indicated a duplication, the precise origin of extra material in 11p would have required considerable trial and error in growing, labeling and hybridizing multiple BAC clones to find those specific to the area of interest. CGH, on the other hand, has the advantage of narrowing the origin of the extra material and localized it to specific bands, 11p14-11p15. Further characterization of the region with locus-specific BAC clones or probes is in progress. To our knowledge, this is the first example of a familial, cytogenetically visible duplication of euchromatin in 11p.

**Chromosome-specific variation in telomere lengths & somatic cell aneuploidy acquired with age: A twin study.** C. Rehder, L. Corey, C. Jackson-Cook. VCU, Richmond, VA.

While we know that telomeres shorten and aneuploidy increases with age, little is known about the relationship between these two phenomena, their tissue and chromosome-specific profiles in normal (non-cancerous) cells, and the influence of genetic and environmental factors on these traits. Thus, to date, we have scored aneuploidy levels in uncultured lymphocytes & buccal mucosa cells using interphase FISH and measured chromosome arm-specific telomere lengths in metaphase chromosomes (using a Q-FISH method) in 38 females [10 MZ & 6 DZ twin pairs] (ages 52-77). In lymphocytes, for all chromosomes examined, loss exceeded gain, with chromosomes 16 (4.7%); 17, 9, 2 & 1 (3.0%) showing the highest loss frequencies. These chromosomes (as well as 7, 12 & X) had significantly lower levels of loss in buccal smears ( $p < 0.001$ ). Interestingly, chromosome 7 showed significantly higher frequencies of gain (up to 6.8%) in buccal mucosa cells compared to lymphocytes ( $p < 0.0001$ ). Although average hypoploidy frequencies were not correlated between tissues, losses of chromosomes 2 and 17 were significantly correlated. Only loss of the X chromosome in lymphocytes showed a significant correlation with age ( $p < 0.01$ ). MZ twin pairs were significantly correlated for aneuploidy frequencies involving chromosomes 9, 16, 17 & X, suggesting an additive genetic effect. For chromosome-specific telomere lengths, differences were seen between females and chromosomes ( $p < 0.0001$ ) with the acrocentric chromosomes (p arms) and 2q having the longest and shortest telomeres, respectively. As expected, mean telomere length decreased with advancing age. For those females having the highest mean levels of hypoploidy ( $> 3.5\%$ ), a significant correlation was seen between increased acquired aneuploidy levels and shortened telomeres ( $p < 0.05$ ). In summary, we determined that acquired aneuploidy frequencies in females are influenced by age, heterochromatin content, and telomere length. Further study of these traits and the genetic and environmental factors that contribute to them will be helpful in understanding susceptibility to change in the human genome, as well as disease onset and progression.

**Familial 6q27 microdeletion detected by high resolution cytogenetics: Report on a family without an MCA/MR syndrome.** *L. Rong*<sup>2,1</sup>, *D. Wolff*<sup>1</sup>, *M. Kral*<sup>1</sup>, *A. LaRosa*<sup>1</sup>, *S. Pai*<sup>1</sup>. 1) Medical University of SC, Charleston; 2) Children's Hospital of Zhejiang University School of Medicine, People's Republic of China.

We evaluated a 14-month-old male for mild developmental delay, hypotonia and subtle facial dysmorphism. He was born by repeat C-section at term weighing 3240 gm. History was negative except for otitis media. Family history showed father with reading difficulty, uncle with hydrocephalus and aunt with congenital hip dysplasia. On physical examination height and weight were normal and the OFC was at the 5th centile. He had large, broad pinnae, broad nasal tip, well developed nasal bridge, mild hypotonia and otherwise normal examination. A consultant found no ocular or retinal abnormalities. CAT/CLAMS tests showed DQ of 83 and 70 for visual motor and speech and language skills, respectively. Diagnostic studies including urine organic acids, metabolic screen, serum amino acids, 7-dehydrocholesterol, very long chain fatty acids, FMR1 gene and abdominal ultrasound were normal. Brain MRI showed subependymal heterotopia in the lateral wall of the right occipital horn and mild hypointensities in the white matter suggestive of periventricular leukomalacia. High resolution chromosomal analysis showed a karyotype 46,XY del(6)(q27). Telomere FISH revealed absence of signal on one #6 homologue. No other structural abnormality was found by FISH with a whole chromosome 6 paint probe. Karyotypic analysis of his parents showed an apparently identical 6q deletion for the father. A del(6)(qTEL) was found by FISH in the father, father's brother and sister. Patient's mother and sister had normal karyotypes. All three adults with deletion 6q27 are high school graduates and gainfully employed. Proband's father achieved a full scale IQ score of 96(90 % confidence interval 93-100) on the Wechsler Abbreviated Scale for Intelligence(WASI). More extensive studies are planned. A review of 38 cases of 6q terminal deletions encompassing band 6q27 showed high incidence of CNS anomalies and mild to severe MR in all cases. The family reported here suggests that microdeletions within 6q27 only may have a more subtle phenotype that does not always include mental retardation.

**Characterization of Novel 4 and 6 Mb LCR-mediated Microduplications Involving the 22q11.2**

**DiGeorge/Velocardiofacial Syndrome Region.** *E.C. Thorland<sup>1</sup>, R.E. Ensenauer<sup>2</sup>, A. Adayinka<sup>1</sup>, H.C. Flynn<sup>1</sup>, D.B. Dawson<sup>1</sup>, J.L. Goldstein<sup>3</sup>, M.T. McDonald<sup>3</sup>, S.M. Jalal<sup>1</sup>.* 1) Lab Medicine and Pathology, Mayo Clinic, Rochester, MN; 2) Medical Genetics, Mayo Clinic, Rochester, MN; 3) Pediatrics, Duke University, Durham, NC.

Greater than 88% of patients with DiGeorge/velocardiofacial (DG/VCFS) syndrome share a common 3 megabase (Mb) deletion of 22q11.2. Low copy repeats (LCRs) have been implicated as mediators of non-allelic homologous recombination which is expected to produce both deletions and duplications of this region in equal proportions. However, although microdeletion occurs with a frequency of 1/4,000 live births, carriers of duplication of 22q11.2 are rarely reported. Utilizing interphase FISH, we have identified 13 cases of 22q11.2 microduplication that were suspected to have DG/VCFS. Clinical features vary widely with some overlap with DG/VCFS. The sizes of the microduplications have been characterized using a combination of overlapping FISH probes and STR (short tandem repeats) markers. These analyses have demonstrated that in addition to the common 3 Mb duplication (the reciprocal event of the common 3 Mb deletion), 4 Mb and 6 Mb duplications are also present in 4/13 and 2/13 patients, respectively. The origin of a 4 Mb duplication in one family with 4 affected members was determined to have occurred in the germline of the maternal grandfather. All of the duplications have a common centromeric boundary in an interval containing an LCR. Similarly, the telomeric boundaries of the 3, 4 and 6 Mb duplications are located within intervals that also contain LCRs. Deletions larger than 3 Mb have not been observed in DG/VCFS patients. Thus, larger deletions corresponding to the reciprocal of the 4 and 6 Mb duplications may not be compatible with life. Since patients were tested based on suspicion of DG/VCFS, the clinical spectrum of this newly recognized syndrome may actually be broader than presently recognized. In addition, the frequency of duplication of 22q11.2 may actually be greater than that of the deletion since there is no apparent selection against duplications that are larger than 3 Mb in size.

**Familial duplication dup(1)(p36.3) with minimal dysmorphism.** *V.S. Tonk<sup>1</sup>, G.N. Wilson<sup>1</sup>, G.V.N. Velagaleti<sup>2</sup>.* 1) Dept Pediatrics, Texas Tech Univ, Lubbock, TX; 2) Dept of Pediatrics, University of Texas Medical Branch, Galveston, TX.

Isolated duplication of the short arm of chromosome 1 are extremely rare with only 11 cases reported, most of them involve proximal and interstitial regions. Duplications of the terminal 1p are even rare with only 1 case reported. Here we report on a family where in an isolated duplication of 1p is observed in three members. The proband is a 15-year-old Caucasian female referred for seizures and mental handicap. Her height and weight were below the 5th percentile. She had difficult temperament and was diagnosed with ADHD. She has minimal dysmorphic features like hypertelorism and malformed ears. She had muscular hypotonia. Chromosome analysis revealed a karyotype 46,XX, dup(1)(p36.3). Her brother, an 8-year-old has similar problems with difficult temperament and ADHD. His height and weight were at the 25th percentile. Other than a down-turned mouth, he had no other dysmorphic features. Chromosome analysis showed similar karyotype 46,XY,dup(1)(p36.3). Subsequent chromosome analysis on the parents showed that the probands mother also has the same chromosome rearrangement. FISH studies with painting, locus specific and sub telomere probes confirmed the duplication. This is the first report of a terminal dup(1)(p36.3) segregating in a family with minimal phenotypic consequences. A complementary deletion involving the same p36.3 region is known to result in a clinically recognizable syndrome with features such as late closing anterior fontanel. Metopic synostosis is reported in duplication of 1p36.3. Such opposing phenotypes associated with deletion and duplication of 1p36.3 prompted speculation that this region contains genes that are involved in suture closure. Interestingly, chromosome 1p36.2 is known to harbor an intriguing gene cluster of about 1 MB including the constitutional neuroblastoma translocation breakpoint. This region consists of locally repeated sequences, thus recombination and unequal crossing over between these repeats can lead to both duplication and deletions. This might be one explanation for the complementary deletion and duplication of 1p36.3 region.

**3:1 segregation of reciprocal translocations: Examples and frequency.** *G.D. Wenger<sup>1,2</sup>, M. Springer<sup>1,3</sup>, Y. Syed<sup>1</sup>, J.F. Atkin<sup>3,4</sup>, J.M. Gastier-Foster<sup>1,2</sup>.* 1) Laboratory Medicine/Cytogenetics, Columbus Children's Hosp, Columbus, OH; 2) Dept. of Pathology, OSU College of Medicine and Public Health, Columbus, OH; 3) Human and Molecular Genetics, Columbus Children's Hosp, Columbus, OH; 4) Dept. of Pediatrics, OSU College of Medicine and Public Health, Columbus, OH.

Unlike single gene disorders, for which the calculation of recurrence risk is generally a straightforward exercise, determination of risk figures for chromosome rearrangements requires characterization of the specific rearrangement and prediction of the mode of segregation for each rearrangement. Balanced autosomal reciprocal translocations are relatively common. While most reciprocal translocations are unique to individual families, empiric data does exist for some situations, notably Robertsonian translocations and the recurring constitutional translocation t(11;22)(q23.3;q11.2). For most others, inspection of the breakpoints and the pachytene configuration which allows maximal alignment of homologous segments is needed for prediction of the mode of segregation. In 3:1 segregation, the unbalanced karyotype includes a supernumerary small derivative chromosome. This type of malsegregation, such as is seen with the recurring constitutional translocation t(11;22), occurs more rarely. In this laboratory, 6 families with t(11;22) were identified during the time period 1993-2002. In addition, 5 families with supernumerary chromosomes due to 3:1 malsegregation were identified; 4/5 of these were maternal in origin. This is in agreement with recent literature suggesting an excess of maternal origin for all unbalanced forms, particularly those resulting from 3:1 segregation. Additional cases were judged to have the potential for 3:1 segregation. An unusual case with t(13;18) is presented. In the ten year period, 201 balanced and 109 unbalanced translocations were identified in this laboratory; 31 of these cases involved 3:1 segregation or indicated a tendency for 3:1 segregation. The cytogenetic mechanism of 3:1 segregation that results in tertiary trisomy is an interesting and relatively uncommon event which should be considered when one of the derivative chromosomes of a reciprocal translocation is very small.

**Submicroscopic Chromosomal Gains and Losses in Patients with Idiopathic Mental Retardation Assessed Using Whole Genome CGH MicroArrays.** *C. Tyson<sup>1</sup>, C. Harvard<sup>1</sup>, JM. Friedman<sup>2</sup>, S. Langlois<sup>2</sup>, SL. Yong<sup>2</sup>, M. Hrynychak<sup>3</sup>, E. Rajcan-Separovic<sup>1</sup>.* 1) Pathology, University of British Columbia, Vancouver, Canada; 2) Medical Genetics, University of British Columbia, Vancouver, Canada; 3) Cytogenetics, Royal Columbian Hospital, New Westminster, Canada.

Idiopathic mental retardation (IMR) is present in 1-1.5% of the population. Many cases of IMR are believed to be caused by small chromosomal deletions or duplications. The resolution of standard cytogenetic techniques is 5-10 Mb, so smaller imbalances remain undetected. We used whole genome microarray CGH with a resolution of 2-4 Mb (Spectral Genomics, Inc.) to investigate potential sub-microscopic chromosomal deletions and duplications in children with IMR and normal karyotypes. A preliminary study was performed to assess the accuracy of the arrays. One normal individual and 5 cases with known chromosomal imbalances were tested. These included: one case with an interstitial deletion of 5q, two siblings with partial monosomy 2q and partial trisomy 17q, one case with a sub-telomeric deletion of 14q and one case of Wilms tumor with multiple chromosomal abnormalities. In all cases the known chromosomal rearrangements were detected with the arrays; 3 of the 5 abnormal cases were tested blindly. Previously-reported polymorphic copy number variants were found for single clones on 6q, 1q, and 17q. No abnormalities were seen in the normal individual. To date, 2 patients with IMR and an apparently normal karyotype at 500-550 band resolution have been tested with microarray CGH. Case 1 - a patient with IMR, microcephaly, mild dysmorphism and hirsutism. A deletion of two adjacent, terminal clones, RP11-58F7 and RP1-3K23, from 7q was demonstrated by CGH array analysis. The size of this deletion is estimated to be 2-4Mb. Case 2 : A duplication of one clone, RP11-143E20, from Xp22.2 was found in a patient with IMR, dysmorphic features and severe constipation. The observed changes will be further assessed using FISH. These preliminary data demonstrate that the 2-4 Mb CGH microarray can confirm visible chromosomal changes as well as detect sub-microscopic abnormalities in mentally-retarded individuals with apparently normal karyotypes.

**Chromosome 2 interstitial deletion [del(2)(q13q21)] associated with hypotonia, mild dysmorphism and hyperviscosity syndrome.** *S. Patel*<sup>1</sup>, *M.J. Macera*<sup>2</sup>, *S. Razvi*<sup>1</sup>, *A. Parekh*<sup>1</sup>, *E. Koenig*<sup>1</sup>, *S. Kleyman*<sup>2</sup>, *A. Babu*<sup>2</sup>. 1) Department of Pediatrics, Long Island College Hospital, Brooklyn, NY; 2) Division of Molecular Medicine and Genetics, Department of Medicine, Wyckoff Heights Medical Center.

A male child was born at 39 weeks gestation by normal vaginal delivery to a 28-year-old mother. His birth weight 2440 gm, length 49.5 cm and head circumference of 33 cm were small for the gestational age parameters. Physical examination demonstrated increased anteroposterior diameter of the skull with molding of the cranial sutures and overriding of the sagittal suture. Mild dysmorphic features included a small bridge of the nose, a thin lower lip with down turned angles of the mouth and a long philtrum. The chest showed widely spaced nipples. There was hypotonia especially of the trunkal muscles with moderate head lag, poor sucking and rooting reflexes and a weak high-pitched cry. MRI revealed symmetric hyperintense T1 weighted extra-axial posterior fossa signal corresponding to a slow flow within the transverse and sigmoid sinuses versus thrombosis. MVR of the intracranial circulation was suggestive of partial thrombosis. The clinical features were also consistent with a hyperviscosity syndrome, with elevated hematocrit and thrombocytopenia. A 6 month follow-up revealed mild developmental delay and a catch up in the physical parameters. Chromosome analysis of peripheral blood at birth, revealed an abnormal 46,XY,del(2)(q13q21) karyotype. Parental chromosome analysis revealed normal karyotypes establishing the de novo origin of the del(2) in the child. Two cases with similar chromosome breakpoints have been reported in the literature (Schinzel 2001). It is hypothesized that both of those cases arose from balanced translocations involving chromosome 2 in one of the parents. It is interesting to note that all three cases expressed very different phenotypes. This case appears to be the first reported case of a del(2)(q13q21) having a de novo origin. Sumption and Barber (2001) have described a case of transmitted deletion (2q13-q14.1) with no phenotype. Based on this, it is possible the major clinical findings in our patient may be contributed by the distal region of the deleted segments i.e. 2q21.

**Detection of a submicroscopic interstitial deletion on 7q31 in a patient with a complex, apparently balanced, t(5;7;6) using CGH arrays.** *E. Rajcan-Separovic*<sup>1</sup>, *B. McGillivray*<sup>2</sup>, *C. Chijiwa*<sup>2</sup>, *C. Tyson*<sup>1</sup>. 1) Dept Cytogenetics, (Pathology), BC Children's Hosp, Vancouver, BC, Canada; 2) Department of Medical Genetics, University of British Columbia, Vancouver, Canada.

We describe the application of CGH array technology to elucidate a complex, apparently balanced translocation in a patient with idiopathic mental retardation (IMR). We used a commercial human CGH array (Spectral Genomics, Inc.), with a 2-4Mb resolution. The patient is a 13 year old male with bilateral cleft lip and palate, hearing loss and IMR. His mother and older sister had learning disabilities. G-banding and multicolor FISH revealed an apparently balanced 3-way translocation involving chromosomes 5, 6 and 7. Chromosomal CGH was performed to investigate whether there was any net loss of DNA as a result of the rearrangement. No abnormality was detected at any of the translocation breakpoints (5p13.2, 6p24, 7q21.1 and 7q21.3), although a deviation of the profile towards loss was seen at band 7q31. However, this deviation fell short of the laboratory's cut off level of 0.8, and was outside the region involved in the translocation breakpoint. CGH array analysis was initiated to clarify this finding and showed a deletion of two adjacent clones, CTB-133K23 and RP11-112P4, mapping to 7q31 and 7q31.3, respectively. These two deleted clones are 4.4Mb apart. FISH with these two clones performed on metaphase chromosomes from the patient confirmed the deletion. In light of this data from the CGH array experiment, the chromosomal CGH experiment was repeated. Once again there was a deviation at band 7q31; however, this time the loss fell above the requisite cut-off level for chromosomal loss, confirming that a very small deletion had occurred at band 7q31. FISH was performed with the two deleted clones on the patient's mother and no evidence of a deletion of either clone was found. This study has demonstrated that the CGH array technique can detect and precisely delineate sub-microscopic chromosome imbalances in patients with complex, apparently balanced chromosomal rearrangements. The new information provided by the CGH array technique will assist in genetic counseling of this family.

**Reproducibility and performance of Vysis GSA300 platform for pre- and post-natal array-based CGH diagnostics.** *E. Pestova, T. Ruffalo, T. Marble, M. Lucas, K. Wilber, W. King.* R&D, Vysis, Downers Grove, IL.

Genosensor<sup>TM</sup>Array 300 (GSA300) is a multiplex platform for array-based comparative genomic hybridization that detects unbalanced genomic aberrations including whole chromosome gains/losses, microdeletions, duplication, and unbalanced sub-telomeric rearrangements. In pre- and post- natal diagnostics, multiplex technologies such as CGH microarrays permit the simultaneous measurement of copy number changes across many genomic loci in a single experiment and require relatively small amount of specimen for analysis. Because of its greater resolution over conventional cytogenetics, it is ideally suited for the detection of small chromosomal aberrations that are associated with multiple disorders ranging from idiopathic mental retardation to micro-deletions syndromes. In the present study, we assessed the analytical performance, reproducibility, and utility of GSA300 arrays for potential application in pre- and post-natal diagnostics. We evaluated the performance of the arrays using DNA extracted from 1 microdeletion cell line (46,XX,del(22)(q11.2q11.2) Di-George) and 4 trisomic cell lines (47, XY, +13, 47, XY, +21, 47,XY,+18, and 47,XY,+2), blood samples from eight apparently normal donors, and amniotic cells obtained from apparently normal male and female pregnancies. In our studies, using DNA isolated from cell lines and normal reference DNA we were able to reliably detect each of the known genomic abnormalities, and achieved an analytical sensitivity of 90%, specificity of 99%, and low user-to-user variation. Using normal blood samples, we demonstrated specificity of 99% and an average CV for the replicate chips for the same specimen of 10%. Amniotic cells grown in culture allowed for accurate and reproducible identification of fetal gender. We have also demonstrated the ability to detect a 14qtel microduplication in one of the amniotic specimens.

**Familial cryptic chromosomal imbalances: assessment by FISH, CGH, m-FISH and array CGH.** *JC. Wang<sup>1</sup>, C. Tyson<sup>1</sup>, B. McGillivray<sup>2</sup>, H. Bruyere<sup>3</sup>, E. Rajcan-Separovic<sup>1</sup>.* 1) Department of Pathology, University of British Columbia, Vancouver, Canada; 2) Department of Medical Genetics, University of British Columbia, Vancouver, Canada; 3) Cytogenetics Laboratory, Vancouver General Hospital, Vancouver, Canada.

We describe two families with two individuals in each family showing an apparently identical chromosomal imbalance and different clinical outcomes. To test the possibility that this may be due to subtle differences in the extent of the chromosomal imbalance we are using a panel of molecular cytogenetic methods including the 2-4Mb resolution array (Spectral Genomics, Inc). Family A includes a baby-boy with multiple congenital anomalies-MCA (right radial and thumb aplasia, dysmorphic features, low sacral dimples, simian crease on left hand) and his father who, apart from autism and developmental delay, has no other clinical features. G banding, chromosomal CGH, sub-telomere testing and m-FISH demonstrated a gain of 2p25.3-ter and loss of the subtelomeric end of 10q in both family members. The CGH array analysis is in process. Family B: Two pregnancy losses from the same patient showed a loss of 2q37.2-qter and gain of 17q25-qter by chromosomal CGH. The pregnancies were terminated at different stages of development- pregnancy 1 terminated spontaneously at 12 weeks, while pregnancy 2 continued until week 34 and resulted in a baby with MCA who died at 24h of life. The mother was found to have a balanced, sub-telomeric t(2;17). The 2-4 Mb resolution array analysis did not demonstrate a difference in the extent of the 2qter loss and 17qter gain between the pregnancies: 7 clones from 2qter were lost and 7 clones from 17qter were gained in both pregnancies. However, the array study delineated the extent of the gain/loss more precisely and indicated that the breakpoint on 2q is more proximal than determined by chromosomal CGH. This suggests that if genomic alternations responsible for the phenotypical differences between these two pregnancy losses exist, they are most likely beyond the resolution of the CGH array we use now. The development of genomic arrays of higher resolution will help in further assessment of these patients.

**Interstitial deletions of proximal chromosome 6q: contribution to the phenotype.** *L. Turner<sup>1</sup>, J.-C. Wang<sup>2</sup>, S.-L. Yong<sup>1</sup>, J. Siegel-Bartelt<sup>1</sup>, E.R. Separovic<sup>2</sup>, P. Eydoux<sup>2</sup>.* 1) Department of Medical Genetics; 2) Department of Pathology, University of British Columbia, Vancouver, Canada.

Interstitial deletions of chromosome 6q have been rarely reported. Three phenotypic groups associated with 6q deletions have been described: group A (q11-q16), group B (q15-q25) and group C (q25-qter). We report on three new cases of proximal deletion of chromosome 6q (group A), further delineating the phenotype of these rare chromosome abnormalities. Case 1 was a newborn female with multiple minor abnormalities, including upslanting palpebral features, abnormal palmar creases, joint laxity and kidney abnormalities. Cytogenetic analysis showed an interstitial deletion of chromosome 6q11-q13, confirmed by CGH analysis. Case 2 shared similar features as case 1, but also had hypotonia, umbilical hernia. Chromosomal analysis allowed the detection of a deletion 6q13-q15. Case 3 was a 6 year-old female with developmental delay, mild hypertelorism, prominent eyebrows and obesity. A small deletion of 6q16.2 was seen on the blood karyotype. Deletions of chromosome 6q11-q16 are characterized by a high incidence of upslanting palpebral fissures and hernias, whereas intra-uterine growth retardation, hypertelorism and upper limb malformations have been described in more distal deletions. A Prader-Willi-like phenotype has also been reported with a deletion 6q15-q21. Our patients, carrying relatively small deletions of chromosome 6q, will help delineate the phenotype of corresponding segmental deletions. Further characterization of the breakpoints using molecular technologies will help the identification of genes contributing to the phenotype of these chromosomal diseases.

**A double cryptic chromosome imbalance is the main factor to explain phenotypic variability in Wolf-Hirschhorn syndrome.** *M. Zollino<sup>1</sup>, R. Lecce<sup>1</sup>, M. Murdolo<sup>1</sup>, I. Mancuso<sup>1</sup>, A. Selicorni<sup>2</sup>, G. Zampino<sup>3</sup>, L. Garavelli<sup>4</sup>, G. Marangi<sup>1</sup>, A. Ferrarini<sup>2</sup>, J.M. Opitz<sup>5</sup>, G. Neri<sup>1</sup>.* 1) Dept Medical Genetics, Univ Catt Sacro Cuore, Rome, Italy; 2) Dept Pediatrics "G.andD.De Marchi, Univ Milano, Italy; 3) Dept Pediatrics, Univ Catt Sacro Cuore, Rome, Italy; 4) Pediatrics Arcispedale S.Maria Nuova, Reggio Emilia, Italy; 5) Division of Medical Genetics, University of Utah, Salt Lake City, USA.

WHS is a MCA/MR syndrome caused by a partial 4p deletion, with a critically deleted region (WHSCR-2) in 4p16.3. Although the severity of clinical presentation usually correlates with the size of the deletion, unexplained phenotypic variability represents an hallmark of this condition. A total of 5 WHS patients with apparent de novo microdeletions (normal chromosomes and hemizygoty of WHSCR-2) presented with a severe phenotype, including major malformations. On further FISH analyses, the actual deletion size resulted to be of 12 Mb (3 cases), 5 Mb and 20 Mb. Additional chromosome segments on the deleted 4p were inferred, and then searched by FISH with subtelomeric probes. A de novo unbalanced t(4;8) translocation was detected in 4 patients and a der(4p;4q) in the last. All the unbalanced t(4p;8p) were maternal in origin, der(4p;4q) was paternal. To verify frequency and specificity of this phenomenon, we investigated yet another group of 20 WHS patients with de novo large deletions (n 13) or microdeletions (n 7) and with straightforward genotype-phenotype correlations. We found that: a) 24% (6/25) of rearrangements were maternal in origin, 76% (19/25) were paternal; b) all the maternally derived rearrangements were de novo unbalanced translocations, which were t(4;8) in five cases, t(4;11) in one case; the 4p deletion interval was of 12 Mb in 4 cases, 5 Mb in one case, and 4 Mb in the last, giving rise to phenotypes of different severity; c) unbalanced translocations caused large deletions mistaken as microdeletions; d) paternally derived rearrangements were isolated deletions, with the only exception of a double intrachromosomal rearrangement. We conclude that a double cryptic chromosome imbalance is the main factor to explain phenotypic variability of WHS.

**AHO-like deletion syndrome: significant refinement of the minimal deleted region at 2q37.3.** *R.C. Trembath*<sup>1</sup>, *M.C. Bonaglia*<sup>2</sup>, *R.C.M. Hennekam*<sup>3</sup>, *M.A. Aldred*<sup>1</sup>. 1) Division of Medical Genetics, University of Leicester, UK; 2) IRCCS E. Medea, Bosisio Parini, Lecco, Italy; 3) Department of Pediatrics and Clinical Genetics, Academic Medical Centre, Amsterdam, The Netherlands.

Deletion of 2q37.3, the telomeric region of chromosome 2q, is associated with an Albright hereditary osteodystrophy-like syndrome. This is characterized by developmental delay, hypotonia, facial dysmorphism and shortening of the metacarpals and metatarsals. Additional features may include obesity, short stature, strabismus, eczema, sparse or coarse hair and autism or other behavioral disturbances. Such deletions have traditionally been studied by conventional cytogenetics, FISH and microsatellite analysis, but with limited resolution. To better define the commonly deleted region, we have developed new assays for systematic assessment of gene dosage across the 2q37.3 region by multiplex amplifiable probe hybridization (MAPH), quantitative real-time PCR and a panel of new microsatellites. The MAPH assay covers 20 known genes and 5 microsatellite loci from 2q37, together with 8 control loci, in two hybridizations. Using these methods, the breakpoints in two previously published cases, a complex duplication/deletion and a deletion patient with classical AHO-like phenotype, were both mapped within a ~0.5Mb region in the vicinity of the *HDAC4* gene. Furthermore, *DERMO-1*, a *TWIST*-related gene involved in bone development, was mapped proximal of these breakpoints and was thus excluded as a candidate for the brachydactyly phenotype. These results are of immediate clinical utility, representing a ~50% reduction of the minimal deleted region and confining it to the most telomeric ~3Mb of 2q. Additionally, MAPH offers a rapid means of high-resolution dosage analysis across the 2q37.3 region that has recently allowed us to exclude major deletion in a patient with an AHO-like phenotype where initial microsatellite analysis was uninformative. Analysis of a wider panel of patients is ongoing to further narrow the critical region and gene(s) responsible for this phenotype.

**Consanguinity and Familial Recurrent Abortion Due To Balanced Translocation (13q;17p) A case report and brief review.** *M.A. Redha, D.S. Krishna Murthy, L.A. Bastaki, S.A. Al-Awadi.* Cytogenetic Laboratory, Kuwait Medical Genetics Center, Maternity Hospital, Kuwait.

**INTRODUCTION AND OBJECTIVES:** Duplication or deletion of chromosomes due to balanced /unbalanced (sporadic/inherited) is one of the major causes leading to multiple congenital anomalies in newborn or to repeated miscarriage. A family (consanguineous) with repeated pregnancy losses in several members of the extended family were investigated to rule out possible chromosomal abnormalities in specific chromosome as the underlying cause for repeated pregnancy losses. **Methodology:** Two young healthy sisters married each to their first cousin were referred to the center with history of repeated pregnancy loss, plus each having healthy children. Metaphase chromosomes of both sisters and their husbands were prepared from short term peripheral blood culture and karyotype analysis was done after (G-banding) by using PS1 power gene karyotyping system. The same study was done for all the children of one family. The other family members were unavailable for study. **Results:** Analysis of 30 metaphases (G-banding) in both healthy sisters confirmed a balanced translocation 46,XX,t(13;17) in all the cells, the same karyotype showed in some of the healthy children suggesting deletion/ duplication of chromosome 13 and 17 is lethal, which confirms maternal origin of chromosomal unbalanced rearrangement. **Conclusion:** Prenatal diagnosis in families having repeated pregnancy losses is extremely important to understand etiology and parental origin very precisely to provide better genetic counseling.

**A patient with DiGeorge-like syndrome and interstitial deletion 10p12.1-p12.31: Refining the critical region responsible for cardiac abnormalities.** S.A. Yatsenko<sup>1</sup>, A.N. Yatsenko<sup>1</sup>, K. Szigeti<sup>1</sup>, P. Stankiewicz<sup>1</sup>, S.W. Cheung<sup>1</sup>, J.R. Lupski<sup>1,2,3</sup>. 1) Department of Molecular & Human Genetics and; 2) Pediatrics, Baylor College of Medicine; 3) Texas Children Hospital, Houston, TX.

DiGeorge syndrome/Velocardiofacial syndrome (DGS/VCFS) is a frequent congenital disorder resulting from abnormal development of the third and fourth pharyngeal pouches. Usually, the phenotype is characterized by a hypoplastic thymus with T-cell deficiency, hypoparathyroidism, conotruncal heart defects, craniofacial dysmorphism, and developmental delay; however, there can be tremendous clinical variability. The submicroscopic deletion within chromosome region 22q11.2 (DGS1/VCFS1) involving *TBX1* is a major cause of DGS/VCFS. A second DGS locus (DGS2) has been described in patients with cytogenetically visible deletions of chromosome 10p. DGS2 is also considered to be a contiguous gene syndrome. Haploinsufficiency of at least two different genes on 10p13-p14 contributes to the DGS2 phenotype. Deletions of 10p14 and mutations in *GATA3* gene are associated with a HDR syndrome (Hypoparathyroidism, sensorineural Deafness, and Renal defects). Deletions proximal to 10p14 are responsible for cardiac and T-cell abnormalities. Recently, the nebulin gene (*NEBL*) was shown to be deleted in two patients with cardiac and craniofacial abnormalities and deletions of 10p12-p13. We present a four-year-old boy with craniofacial dysmorphism and developmental delay. Echocardiogram revealed trivial mitral and tricuspid regurgitation without gross structural abnormalities. MRI of the head showed aqueductal stenosis, mild compensated hydrocephalus, and Chiari I malformation. High-resolution chromosome analysis at 850-band resolution revealed interstitial deletion del(10)(p12.1;p12.32). To characterize the deletion breakpoints we performed FISH analysis using 27 BAC clones. Our data demonstrate a ~5.5 Mb deletion, the smallest one reported to date. Interestingly, the BAC clone RP11-56H7, containing *NEBL* was deleted in our patient. We correlate the clinical manifestations with deletion size in our patient and compare it with previously reported cases. Our data potentially narrow down the DGS2 critical region.

**Paternal balanced translocation, t(6;7)(p21.3;q34) in a couple with recurrent abortions: last of them with trisomy**

**16.** *C. Uria Gomez*<sup>1, 2, 4</sup>, *G. Arteaga Ontiveros*<sup>2</sup>, *E. Blanco Aguirre*<sup>3</sup>, *R. Garcia Cavazos*<sup>1</sup>, *D. Mayen Molina*<sup>1</sup>. 1) Depto de Genetica Inst. Nal. de Perinatologia, Mexico DF, Mexico; 2) Genecon Diagnostico Laboratorio, Toluca, Mexico; 3) Hosp. General Adolfo Lopez Mateos Toluca, Mexico; 4) Fac. de Quimica UAEM, Toluca Mexico.

The rate of spontaneous abortions in balanced translocation carriers was found to be 25-50%, and is a consequence of formation of gametes with unbalanced chromosomes. A healthy non consanguineous young couple was referred for cytogenetic analysis. They had four recognized spontaneous abortions and no live-born children. First, a sample specimen from a 9 weeks gestation abortion (last one out of four) was received for chromosome analysis. Twenty metaphases from four primary cultures were analyzed and the results showed trisomy 16 in all of them. There is not cytogenetic information about three previous abortions. Chromosomal analysis in the wife showed 46,XX. The Karyotype in the husband surprisingly showed 46,XY,t(6;7)(p21.3;q34). The translocation was further confirmed by FISH using whole chromosome paints for 6 and 7. We ignore the trisomy 16 parental origin in the last abortion, but trisomies are generally due to a maternal meiosis error. We observed again trisomy 16 metaphases and translocation was not present. This is an unusual translocation associated with fetal loss and aneuploidy. To our knowledge there is only one report about translocation (6;7), but this is (6q;7q). We ignore if some kind of segregation is preferential for this paternal translocation, but the fact that no malformed live births and no living child were observed, implies that the offspring gametes should have an unbalanced chromosomes. Genetic counseling such as prenatal diagnosis including FISH on interphase spermatozoa, should also be considered in a future pregnancy.

**An association between meiotic recombination and advancing maternal age for trisomy 21.** *S.L Sherman<sup>1</sup>, E. Feingold<sup>2</sup>, N.E. Lamb<sup>1</sup>*. 1) Human Genetics, Emory Univ., Atlanta, GA; 2) Human Genetics, Univ. of Pittsburgh, Pittsburgh, PA.

Advancing maternal age has long been identified as the primary risk factor for human chromosome trisomy. For specific chromosomes, altered patterns of meiotic recombination have also been associated with an increased risk of trisomy. For trisomy 21, these susceptible patterns include exchange near the centromere or single, telomeric exchanges. To date, however, no relationship has been found between these patterns on chromosome 21 and maternal age. As part of an ongoing study of trisomy 21 (ts21) we examined the recombination status of 562 ts21 cases, all of maternal origin. The cases were genotyped at STR markers spanning chromosome 21 and divided into groups based upon maternal age at the time of the trisomic birth (<29, 29-34, >34 years of age). Tetrad analysis was used to infer the meiotic exchange patterns for each group. Achiasmate tetrads accounted for 39%, 21% and 28%, respectively, of each age population. For those tetrads that participated in exchange, the inferred location of the exchanges varied between the groups in an age-dependent manner. Among the youngest group, 62% of nondisjoining oocytes with exchange had susceptible exchange patterns. These patterns were less frequent in the mid (44%) and older (33%) age groups, which instead contained increased proportions of stable, medially located exchange. These data support theories that suggest the fidelity of meiotic processes may be age-dependent. Oocytes of younger women, with fully functional meiotic apparatus and/or robust ovarian environment, are able to properly resolve all but the most susceptible exchange patterns. As women age, however, meiotic mechanisms erode, making it difficult to resolve even stable exchange events. As a result, nondisjunction rates would increase among older women, regardless of the location or stability of meiotic exchanges. These studies provide the first link between maternal age and the molecular events of meiosis, and suggest that stable chiasmate patterns can provide some initial protection for the tetrad against maternal-age related factors that cause the meiotic degradation and lead to nondisjunction.

**Application of array CGH in clinical genetics.** *M.R. Speicher<sup>1</sup>, H. Fiegler<sup>2</sup>, C. Fauth<sup>1</sup>, P. Carr<sup>2</sup>, J. Kraus<sup>1</sup>, N.P. Carter<sup>2</sup>.* 1) Institute of Human Genetics, Technical University Munich and GSF Neuherberg, Munich, Germany; 2) Wellcome Trust Sanger Institute/Cancer Research UK Genomic Microarray Group, Hinxton, Cambridge, CB10 1SA, United Kingdom.

High-resolution screening tools, such as array-CGH, for detecting small imbalances will become an indispensable tool in clinical genetics. Here, we report several cases, which were analyzed with array-CGH. Array CGH was performed on the recently developed 1 Mb large insert clone array (Fiegler et al. [2003] *Genes Chrom Cancer* 36:361-374). This array consists of about 3,500 clones selected from the published Golden Path which are spaced at approximately 1-Mb intervals across the genome. In one case with an additional marker chromosome derived from chromosome 21, array-CGH readily pinpointed the breakpoint to chromosome band 21q21.1. Array-CGH was also applied to two cases with complex translocations. In the first case, a complex translocation involving four chromosomes and 9 breakpoints was identified in both a mother and her daughter. While the mother has a normal phenotype, the daughter has a moderate mental retardation. To facilitate breakpoint mapping the translocation chromosomes were isolated by flow sorting and hybridized to the 1 Mb BAC array. This provided localization of all breakpoints to within a resolution of 1 Mb. Furthermore, array-CGH of the DNA from the mother and the daughter revealed unexpectedly a deletion on a chromosome which was not involved in the complex translocation. In another case, 7-fluorochrome M-FISH unraveled in a two year old child with multiple dysmorphic features the karyotype: 46,XX,der(5)t(5;14),der(6)t(6;8),der(8)t(5;8;5;8;6;2),der(14)t(8;14). However, we were unable to identify any genomic imbalance, which was expected because of the phenotype of the patient. Array-CGH readily identified 4 different deletions, each with a size of smaller than 7 Mb. These deleted fragments contain 14 different known genes which probably contribute to the patient's phenotype. In conclusion, the application of these novel technologies will unravel the cause of many currently unexplained cases with mental retardation and dysmorphic features.

**Meiotic recombination in the 22q11.2 deletion syndrome.** *S.C. Saitta, S.E. Harris, D.A. Driscoll, D. Conforto, T.H. Shaikh, M.K. Maisenbacher, D.M. McDonald-McGinn, E.H. Zackai, J.M. Yersak, P.K. Chakraborty, B.S. Emanuel.*  
Division of Genetics, Children's Hospital of Philadelphia, Philadelphia, PA.

Detectable deletions of chromosome 22q11.2 are found in almost 90% of patients with DiGeorge/velocardiofacial syndrome (DGS/VCFS). The deletion is estimated to occur at a frequency of 1/3,000 live births, indicating a high de novo mutation rate. Approximately 10% of the deletions are transmitted from an affected parent. The presence of chromosome-specific low copy repeats (LCRs) in the 22q11 deletion interval predisposes the area to non-homologous recombination that could lead to the frequent occurrence of this microdeletion syndrome. To further examine such a mechanism, we traced the grandparental origin of regions flanking the deletion in 20 three-generation families. By FISH the size of these de novo deletions was determined to be the most commonly occurring, standard 3 Mb deletion. Haplotype reconstruction was consistent with an interchromosomal recombination event in 19/20 families. In contrast, the normal allele transmitted in these same families showed crossovers in 2/15 informative meioses, consistent with the rate expected for the physical distance. We also examined the parental origin of the deletion in 62 de novo standard deletions (including these patients) and found no bias. Further, we examined 22 patients and their parents by FISH for evidence of an inversion of chromosomal material around the LCRs flanking the standard 3 Mb deletion and the smaller 1.5 Mb deletion and found none. Eight non-deleted patients with a DGS/VCFS phenotype were also shown to not have an inversion. Our data are consistent with significant interchromosomal exchange events in the 22q11 genomic region that likely relates to the frequency of its deletion. This type of exchange occurs more often than those seen for chromosomes 7q11, 17p11, and 17q11 implying a difference in the meiotic behavior of chromosome 22.

**Paracentric inversions (PAI) and associated risks: A case report of a PAI carrier with two recombinant offspring.** *M.A. Springer<sup>1,2</sup>, J.M. Gastier-Foster<sup>1,3</sup>, L. Erdman<sup>1</sup>, J. Gordon<sup>1</sup>, B. Hamelberg<sup>1</sup>, C. Kulmer<sup>1</sup>, C. Deeg<sup>1</sup>, J. Labanowska<sup>1</sup>, R. O'Shaughnessy<sup>4</sup>, G.D. Wenger<sup>1,3</sup>.* 1) Laboratory Medicine, Columbus Children's Hosp, Columbus, OH; 2) Human and Molecular Genetics, Columbus Children's Hosp, Columbus, OH; 3) Dept. of Pathology, OSU College of Medicine, Columbus, OH; 4) Division of Maternal-Fetal Medicine, The Ohio State University.

Chromosome rearrangements occur commonly in humans, with an estimated incidence of 1/500-1/1,000 liveborns. Inversions are commonly observed and result from two independent breaks in one chromosome, with subsequent inversion of the interstitial segment. While pericentric inversions (PEI) are associated with a greater risk for viable recombinants, paracentric inversions (PAI) have generally been believed to be associated with a lower risk, with the majority of carriers identified incidentally. Recent reviews in the literature, however, have reported viable recombinant offspring from PAI carriers, suggesting that PAI may be associated with an increased risk for abnormal recombinants. We present a case of a 31-year old G3P1 female who presented at 25 weeks gestation with dilated renal pelvises in the fetus. Family history was significant for a 5-year old daughter with developmental delay, significant speech delay, growth hormone deficiency, severe hypotonia, and borderline microcephaly. Amniocentesis revealed an interstitial deletion in the short arm of chromosome 8. Parental karyotypes were obtained, demonstrating a paracentric inversion involving the short arm of chromosome 8 in the mother. Chromosome analysis was subsequently performed on the 5-year old sibling and revealed the same interstitial deletion of chromosome 8 as that found in the fetus. This case represents a rare report of a PAI carrier with two recombinant offspring.

**Unusual inverted duplication of 4p in conjunction with a balanced translocation in a 15-year-old girl with a mildly affected phenotype.** *J. Winters*<sup>1</sup>, *J. Cordell*<sup>2</sup>, *A. Grix*<sup>2</sup>. 1) Kaiser Permanente Northern California, Regional Cytogenetics Laboratory, San Jose; 2) Department of Genetics, Sacramento.

A 15-year-old girl was seen for a genetics evaluation because of her learning handicaps. Prenatal, birth, and family histories were unremarkable. She walked at 19 months and had delayed language development. At birth, she was noted to have bilateral inguinal hernias, which were repaired at 7 weeks of age. Her IQ is reported to be 81. During this evaluation, she was found to be non-dysmorphic with several nevi and learning disabilities. Karyotype analysis was ordered to rule out XXX syndrome. Routine chromosome analysis revealed two apparently independent rearrangements. One chromosome 4 had an abnormal short arm, with extra material inserted interstitially. This same chromosome 4 was also involved in an apparently balanced reciprocal translocation with a chromosome 10. The 4p rearrangement was most compatible with an intra-arm (or paracentric) inverted insertion, resulting in trisomy for the p15.2->p16 segment. FISH was performed with probes for the Wolf-Hirshhorn critical region at 4p16 and the 4p subtelomeric region (WHS and D4S3359, respectively, both from Vysis, Inc.). Both displayed two signals on the der(4) (one in the middle of the short arm and one at the p terminus). The karyotype was determined to be 46,XX,der(4)ins(4)(p15.2p16p15.2)t(4;10)(q25;q23.2),der(10)t(4;10).ish der(4)(4ptel++,WHS++). The mother's karyotype was studied and found to be normal; the father was not available for analysis. Duplications of the terminal regions of chromosomes are usually inverted duplications, thought to arise from a terminal two-strand break, followed by a U-type exchange and breakage between the centromeres (Hoo et al, 1995), leading to both partial trisomy and partial monosomy. In contrast, this case involves a same-arm inverted insertion, leading only to partial trisomy. The presence of two breakpoints on chromosome 4 raises the question of a possible interchromosomal effect.

**Identification of additional cryptic rearrangements in cytogenetically abnormal patients by a CGH microarray chip designed for clinical diagnosis.** A. Patel<sup>1</sup>, W. Yu<sup>1</sup>, C.A. Shaw<sup>1</sup>, L. Cooper<sup>1</sup>, M. Patel<sup>1</sup>, M. Fishman<sup>2</sup>, C. Bacino<sup>1</sup>, S.W. Cheung<sup>1</sup>, A. Beaudet<sup>1</sup>. 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pediatrics-Neurology, Texas Children Hospital, Houston, TX.

Preliminary validation of a CGH microarray chip designed for clinical diagnosis of genetic disorders that can be routinely screened by FISH analysis found additional cryptic rearrangements while confirming the original cytogenetic abnormality in 2 out of 11 patients studied thus far. Patient 8 had an inv dup(8)(p11.23p23.1) cytogenetically and was confirmed to be all chromosome 8 material by whole chromosome 8 paint, and to be deleted for the 8p telomere by FISH analysis. Array CGH analysis confirmed loss of the 8p telomere and found a gain of the 18q telomere. Subsequent FISH analysis with the 18q telomere found the inv dup 8 chromosome to have captured the 18q telomere. This is a second case where an inv dup 8 chromosome has been stabilized by the 18q telomere. Patient 6 had a del(13)(q33.2) karyotype and all telomere FISH analysis with the Vysis telomere probe panel found no other abnormalities. Array CGH analysis confirmed the 13q telomere deletion but also identified a 22q telomere loss. The array 22q telomere clone is distal to the Vysis 22q telomere probe and again FISH analysis confirmed the 22q telomere deletion. Our preliminary data suggests that cytogenetically abnormal karyotypes may also harbor cryptic rearrangements that could go undetected and therefore the use of array CGH together with classical cytogenetics can provide a more accurate and sensitive clinical diagnostic screen for genetic disorders.

Program Nr: 834 from 2003 ASHG Annual Meeting

**CONFLICTING RESULTS BETWEEN PRENATAL INTERPHASE FISH & G-BANDED CHROMOSOME DIAGNOSES;IMPLICATIONS FOR CLINICAL DECISIONS.** *PalD. Singh-Kahlon, I. Gadi, J. Tepperberg, P. Mowrey, L. Wisnewski, S.K. Griffin, C.J. Bullen, P. Papenhausen.* PAL D. SINGH-KAHLON, CYTOGENETICS, LabCorp Inc,1912 ALEXANDER DR,Res Triangle Park, NC.TEL:800-533-0567x3403.

**SUMMARY:**We report a PRENATAL INTERPHASE FISH study on amniotic fluid showing an apparent TRISOMY 18 hybridization pattern. Although ultrasound findings were normal, the couple was counseled for the possibility that their fetus had TRISOMY 18. The subsequent G-banded chromosomal study showed an apparently NORMAL KARYOTYPE. A possible mix up in tube labeling, culture set up, or in the prenatal FISH preparation were all ruled out. The subsequent CHROMOSOMAL FISH analysis showed an extra hybridization of pericentromeric alpha satellite DNA signal, inserted into the proximal long arm of chromosome 9. Parental FISH studies revealed the father to be the carrier of the same rearrangement observed in the fetus. These results emphasize that no clinical decisions regarding ongoing pregnancies should be made, unless an abnormal karyotype and/or abnormal ultrasound findings confirm the FISH results.

**Cloning of the breakpoints of a de novo inversion of chromosome 8 inv (8)(p11.2q22) in a patient with Ambras syndrome.**

*M. Tadin-Strapps*<sup>1</sup>, *D. Warburton*<sup>2,3</sup>, *F. Baumeister*<sup>6</sup>, *G. Swergold*<sup>4</sup>, *S.G. Fischer*<sup>5</sup>, *J. Yonan*<sup>5</sup>, *T.C. Gilliam*<sup>2,5</sup>, *A.M. Christiano*<sup>1,2</sup>. 1) Dermatology, Columbia Univ, New York, NY; 2) Genetics and Development, Columbia Univ, New York, NY; 3) Pediatrics, Columbia Univ, New York, NY; 4) Medicine, Columbia Univ, New York, NY; 5) Columbia Genome Center, Columbia Univ, New York, NY; 6) Dr. v. Haunersches Kinderspital der Universitat Munchen, Germany.

Ambras syndrome (AMS) is a unique form of universal congenital hypertrichosis. In patients with this syndrome the whole body is covered with fine, long hair except for the areas in which normally no hair grows. Minor facial dysmorphism and dental anomalies, including retarded first and second dentition and absence of teeth have been reported as well. Cytogenetic abnormalities of chromosome 8 have been reported in two isolated Ambras syndrome patients. The presence of a common breakpoint in 8q22 in both of these cases suggests the presence of a candidate gene(s) in this region of chromosome 8. In order to precisely determine the nature of the rearrangement in a case of Ambras syndrome, we have used Fluorescent In Situ Hybridisation (FISH) with YAC and BAC clones from 8q22-q24. We have generated a detailed physical map across the inversion breakpoint interval, cloned and sequenced inversion breakpoints in this patient, and identified transcripts in the vicinity of the breakpoints. Genomic sequence analysis of the breakpoint interval revealed that the inversion does not disrupt a gene and suggests that the phenotype is caused by a position effect. Interestingly, the breakpoints occurred within a Tigger1 element on the 8p arm, and the LINE1 element on the q arm. We are currently analysing expression of the genes that map in the vicinity of the inversion breakpoints.

**Cytogenetic and Hematologic investigations of patients with severe chemical injuries after exposure to Mustard Gas during Iran-Iraq conflict.** *M. Shafa Shariat Panahi*<sup>1,3</sup>, *M. Khazab*<sup>2</sup>, *Y. Seyedena*<sup>3</sup>. 1) Genetics Clinic, NRCGEB, Tehran, Iran; 2) Genetics Research Center, Razi Institute, Karaj, Iran; 3) Biology Department, Shahid Beheshti University, Tehran, Iran.

Sulfur mustard (SM) is a potent alkylating agent with mutagenic properties. It has been widely used in Iran-Iraq conflict. This study assessed the impact of this agent on the hematologic parameters and chromosomal aberrations (CA) in the peripheral blood of severely-injured Iranian combatants. Twenty five patients with severe lung and eye injuries and ten controls were included in the study. The subjects of control group were healthy volunteers matched for sex and age. The lymphocytes were cultured by conventional culture methods. Hematologic parameters including CBC, Platelets, Blood index and peripheral blood films were studied. Twenty five well-spread metaphases were scored for each sample for any chromosomal aberrations. Two groups were compared with statistical methods including t-test and chi square test. We found that the mean Hb, HCT, WBC, Platelets, MCV and lymphocyte numbers were different in two groups ( $P < 0.001$ ). Patients and control group showed differences in the percentage of metaphases containing at least one chromosomal aberration. This study showed that there is a direct correlation between SM exposure and hematological as well as cytogenetic anomalies in these patients.

**Is Telomere Length Associated with Trisomy Risk in Humans?** *J. Gair<sup>1</sup>, E. Chavez<sup>3</sup>, M. Stephenson<sup>4</sup>, S. Langlois<sup>1</sup>, P. Lansdorp<sup>2,3</sup>, W. Robinson<sup>1</sup>*. 1) Department of Medical Genetics; 2) Division of Hematology, Faculty of Medicine, University of British Columbia; 3) BC Cancer Agency, Terry Fox Laboratory; 4) Department of Obstetrics and Gynaecology, BC Children's and Women's Hospital, Vancouver, BC, Canada.

Trisomy is a major cause of reproductive failure in humans and is generally due to an error in maternal meiosis. Little is known of its etiology other than it is strongly associated with maternal age. Structural aspects of chromosomes, such as telomeres, may also be important risk factors. Telomeres are important in chromosome pairing and decreased length is associated with increased somatic nondisjunction. We hypothesize that individuals starting life with relatively short telomeres or with lower levels of telomerase activity in early development may have relatively short telomeres in both germ cells and somatic cells and be at increased risk of meiotic nondisjunction. To determine whether shorter telomere length is associated with increased risk of trisomy, telomere length was evaluated in 10 women who had experienced at least one trisomic pregnancy and 9 similarly aged controls with no history of trisomy. Quantitative FISH was performed on metaphase chromosomes from peripheral lymphocytes, using peptide nucleic acid (PNA) probes to the telomeres of all chromosomes. The fluorescence signal from the probes was quantified. The average telomere length of 3.35kb (2.48 - 4.27 kb) in the patient group (N=10) was significantly shorter than the 4.04kb (2.72 - 6.17 kb) observed in controls (N=9) (p-value = 0.05, t-test). Although these preliminary results support our hypothesis, additional studies are underway to confirm this association with a much larger sample size and determine whether this is a primary cause for the increased risk of meiotic nondisjunction or simply an indicator of premature ovarian aging in women presenting with a trisomic pregnancy.

**Reconstruction of 3D-spatial structure of mammalian centromere.** *Y.C. Li<sup>1</sup>, S.J. Liao<sup>2</sup>, L.J. Hsieh<sup>2</sup>, C.C. Lin<sup>1,2</sup>.* 1) Department of Life Sciences, Chung Shan Medical University, Taichung, Taiwan; 2) Department of Medical Research, China Medical University Hospital, Taichung, Taiwan.

Centromere plays a pivotal role during mitosis and meiosis. Malfunctioned centromere would result in aneuploidy that is associated with disorders such as spontaneous abortion, birth defect and some neoplasia. In order to unveil the enigma of aneuploidy, it is an important and necessary to understanding the molecular architecture and composition of centromere. Interestingly, a functional conserved centromeric domain is made up of the diverse repetitive centromeric DNAs and highly conserved kinetochore proteins. Does a various centromeric satellite DNA be organized into a specific structure to associate with conserved kinetochore proteins in mammals? The simultaneous immunofluorescence and FISH study showed that the satellite II and IV were found to co-localize in muntjac centromere and to associate with kinetochore. Furthermore, the 3D- FISH and immunofluorescence were carried out on the polyacrylamide embedded, formaldehyde fixed cells using either biotinylated satellite II probe and digoxigenin-labeled satellite IV probe or one of them and CREST serum. The images acquired from the different Z-axial sections were deconvolved and reconstructed. The reconstructed 3D image showed that the CENP immunofluorescence signals parallels along with centromere whereas both satellite II and IV signals of X+3 centromere are organized into a spiral structure with four turns, each of which showed six fluorescence signals. The spiral structure may be to present centromeric chromatin to the exterior of the chromosome, where it can mediate kinetochore assembly and interactions with the spindle. This study was supported by grants from National Science Council (NSC 91-2320-B-040-037) and National Health Research institute (NHRI-EX92-9207 SI).

**Early-Replicating Human Centromeres.** *Y. Zou, W. Wright, J. Shay.* Cell Biology and Neuroscience, UT Southwestern Medical Center, Dallas, TX.

Heterochromatin, which has a condensed structure and rarely transcribed, usually replicates in late S phase. Here, we found that human heterochromatic centromeres replicate in early S phase. Human BJ fibroblasts had been labeled with 5-bromodeoxyuridine (BrdU) at hourly intervals before they approached to G2 phase, then they were arrested at metaphase with colcemid, and examined the centromere replication profile by strand-specific fluorescence in situ hybridization. Since BrdU labeled DNA strands become selectively labile to UV light and ExoIII treatment, they can be gotten rid of by a six-step treatments. Based on the principle of strand-specific in situ hybridization, a single-stranded centromeric probe can only bind to one of the two sister chromatids of a metaphase chromosome at a centromere locus. If the time point of centromere replication matches BrdU being made available to a replicating chromosome, the strand-specific FISH pattern will be observed. Conversely, if centromere replication is early or late when BrdU is made available to a replicating chromosome, the typical FISH labeling pattern will be observed. Therefore, the timing of centromere replication can be determined by chromosome orientation fluorescence in situ hybridization using dual color labeled strand-specific centromeric probes. This is the first report demonstrating that the majority human centromeres (>90%) are replicating in early S phase. Thus, early-replicating, rather than late-replicating, is a fundamental property of replication timing of human centromeres.

**The donor chromosome breakpoint for a jumping translocation is associated with large low-copy repeats in 21q21.3.** *P. Stankiewicz<sup>1</sup>, S.W. Cheung<sup>1</sup>, C.J. Shaw<sup>1</sup>, R. Saleki<sup>1</sup>, K. Szigeti<sup>1</sup>, J.R. Lupski<sup>1,2,3</sup>.* 1) Depts of Molecular & Human Genetics; 2) Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Children Hospital, Houston, TX.

Jumping translocations (JTs) are very rare chromosome aberrations, usually identified in tumors. We report a constitutional JT between donor chromosome 21q21.3-qter and recipients 13qter and 18qter, resulting in an ~ 15.5 Mb proximal deletion 21q in a girl with mild developmental delay and minor dysmorphic features. Using fluorescence in situ hybridization (FISH) studies, we identified an ~ 550 kb complex inter- and intra-chromosomal low-copy repeat (LCR) adjacent to the 21q21.3 translocation breakpoint. On the recipient chromosomes 13qter and 18qter, the telomeric sequences TTAGGG were retained. Genotyping revealed the deletion was of maternal origin. We propose that genome architecture involving LCRs may be a major mechanism responsible for the origin of jumping translocations.

**Trisomy 3q secondary to a terminal deletion/generation of a mirror-image analphoid marker in a neonate. P.**

*Papenhausen<sup>1</sup>, I. Gadi<sup>1</sup>, J. Tepperberg<sup>1</sup>, P. Mowrey<sup>1</sup>, P. Singh-Kahlon<sup>1</sup>, L. Wisniewski<sup>1</sup>, D. Goodwin<sup>2</sup>.* 1) Dept Cytogenetics, Labcorp of America, Res Triangle Pk, NC; 2) Naval Medical Center, Portsmouth, VA.

A 23 day old female was referred to rule out Turner syndrome by cytogenetics and FISH. The phenotype included venticulomegaly with a VSD and a bicuspid aortic valve. There was a prominent forehead, large fontanelles, broad nasal bridge, and retrognathia. Cytogenetic results from lymphocytes revealed 47,XX,del(3)(q23),+mar in all cells. Although there was an apparent centromeric constriction near one end of the marker, high resolution suggested mirror-image duplication of the deleted 3q23->qter region. An analphoid marker was anticipated due to the frequent inverted duplication structure of that class of marker. The acentric nature was confirmed by FISH. Two distal subtelomere 3q FISH signals confirmed the duplication and the net trisomy for 3q23->qter. The neocentromere constriction was estimated at band 3q27 on one side of the duplicate. Analphoid markers with neocentromeres have tended to originate from limited regions of the genome with frequent hot spots on 3q and 13q. Those that are generated from terminal deletions form isochromosomes while those that are interstitially excised form rings. The isochromosomes are often asymmetric due to off center positioning of the neocentromere. The mechanism of formation of active kinetochores and the necessary molecular sequence remains largely unknown. This is the first such report of an excision based analphoid marker from the long arm chromosome 3 hot spot region.

**An innovative molecular cytogenetics method: Fluorescence in Situ Hybridization of chromosomes in suspension applied to medical genetics.** *Z. Chen*<sup>1</sup>, *J. Kaminsky*<sup>2</sup>, *J.N. Lucas*<sup>2</sup>. 1) Dept Pediatrics/Cytogenetics, Univ Utah Sch Medicine, Salt Lake City, UT; 2) A. L. Tech Biomedical, Inc., Arlington, VA.

Classical FISH is performed on slides and has a detection sensitivity of 1 in 100-1000 cells. PCR is very sensitive, but lack of very accurate quantitation still is a problem. By contrast, an innovative approach, which we have been developing, will greatly enhance accurate and quantitative detection of chromosomal aberrations and has a potential detection sensitivity of 1 in 1,000,000 cells. Our techniques include a novel approach of hybridizing chromosomes in suspension with fluorescently-labeled regular DNA probes or repetitive sequences depleted DNA probes, in combination with a flow cytometric method of analysis or magnetic sorting, in order to sensitively, precisely and rapidly quantify chromosomal translocations and rearrangements. The repetitive sequences depleted DNA probes are generated through our novel two-step method that includes subtracting hybridized biotin-labeled repetitive-sequence DNA complex with phenol and chloroform and a second subtraction using avidin labeled magnetic beads. The unique sequences are then recovered using our dual unique sequence primers. Our pilot experiments have demonstrated the great efficacy of the method in depleting repetitive sequences. In suspension hybridization, our method allows recovery of large numbers (46-73%) of isolated individual chromosomes post hybridization. The well-preserved morphology of hybridized chromosomes allowed rapid detection of exchange chromosome aberrations and counting of chromosomes. The utility of our method for cancer genetics was demonstrated using a probe for the C-MYC oncogene on 8q24. After hybridization in suspension, free floating interphase cells showed two distinct signals and suspended metaphase chromosomes showed bright signals on 8q. Our revolutionary methods offer a new tool to assay chromosomes and DNA and provide the possibility to develop new techniques for sorting chromosomes based on FISH signals, for early detection and screening of genetic diseases and cancer, and for bulk measurement of both balanced and unbalanced chromosomal rearrangements post exposure to mutagens.

**A Strategy for Detecting Submicroscopic Deletions in Patients with Holoprosencephaly using FISH.** *C. Bendavid*<sup>1</sup>, *B. Haddad*<sup>2</sup>, *M. Muenke*<sup>1</sup>. 1) Medical Genetics Branch, NHGRI/NIH/DHHS, Bethesda, MD; 2) Georgetown University Medical Center, Washington, DC.

Holoprosencephaly (HPE) is the most common structural anomaly of the developing forebrain in humans (1:250 during early embryogenesis and 1:10,000 at birth). The etiology of HPE is extremely heterogeneous with known teratogens and genetic factors such as visible cytogenetic anomalies (in 25-50% of newborns) and gene mutations (in 20-25% of chromosomally normal infants). Cytogenetic deletions have helped define HPE minimal critical regions and identify HPE genes: 7q36 (Sonic Hedgehog: *SHH*, 13q32 (*ZIC2*, 2p21 (*SIX3*), and 18p11.3 (TG Interacting Factor: *TGIF*). *DISPATCHED* in 1q14 and *HNF3beta* in 20p11.21 are currently studied as HPE candidate genes. Based on the known HPE-associated cytogenetic anomalies we hypothesize that submicroscopic deletions can lead to HPE. To test this hypothesis we have initiated a study of 100 lymphoblastoid cell lines from unrelated HPE patients without detectable chromosomal alterations by conventional cytogenetic karyotyping and no known mutations in the six previously identified and/or putative HPE genes. We developed a panel of six BAC probes for FISH analysis including the genes for *SHH*, *ZIC2*, *TGIF*, *SIX3*, *HNF3beta*, and *DISPATCHED* from the human RP11 and RP4 libraries (CHORI), using public databases (NCBI, ensembl! and UCSC genome browsers). Each probe was individually mapped to normal human chromosomes to confirm its correct location. The presence of the gene of interest in the BAC clone was confirmed by PCR with primers flanking 2 known gene exonic amplicons. Finally, we set up a multilocus FISH panel to visualize all six probes in a single hybridization. We have validated this panel by using samples from HPE patients with known deletions for the genes in this panel and samples from normal donors in a blinded fashion. To date, we have completed the analysis in 30 HPE patients. This preliminary study did not show any abnormalities using this HPE FISH panel. FISH analysis of additional HPE patients is now in progress, to determine if submicroscopic deletions in known HPE genes contribute to the etiology of HPE.

**Two unrelated cases of Xp21 contiguous gene deletion syndrome: rapid characterization of the deletion using**

**QMPSF.** A. Goldenberg<sup>1</sup>, V. Drouin-Garraud<sup>1</sup>, S. Baert<sup>1</sup>, S. Marret<sup>2</sup>, C. Kolly<sup>3</sup>, C. Vanhulle<sup>2</sup>, C. Forget<sup>4</sup>, E. Bessenay<sup>1</sup>, D. Recan<sup>5</sup>, M-A. Gougerot-Pocidal<sup>6</sup>, T. Frebourg<sup>1</sup>, P. Saugier-veber<sup>1</sup>. 1) Department of Medical Genetics, Rouen University Hospital, France; 2) Department of Neonatology, Rouen University Hospital, France; 3) Department of Paediatrics, Rouen University Hospital, France; 4) Department of Paediatrics, CHG d'Elbeuf, France; 5) Laboratory of Biochemistry and Molecular Genetics, Hpital Cochin, Paris; 6) Laboratory of Haematology and Biologic Immunology, CHU Bichat-Claude Bernard, Paris, France.

The Xp21 contiguous gene deletion syndrome includes Duchenne muscular dystrophy, congenital adrenal hypoplasia, glycerol kinase deficiency, MacLeod syndrome, and X-linked chronic granulomatous disease, involving the DMD, DAX/NROB1, GK, XK and CYBB genes, respectively. We report here on two unrelated cases of Xp21 deletions. The first patient presented at birth with hypotonia and salt wasting syndrome. Polyhydramnios was noted during pregnancy. Elevated CPK and hypertriglyceridemia prompted us to consider the diagnosis of glycerol kinase deficiency associated with Duchenne muscular dystrophy. The clinical follow-up included developmental delay, short stature and mild dysmorphic features. The second patient presented at the age of 10 months, with severe hypotonia and recurrent subcutaneous staphylococcus infections, failure to thrive and mild dysmorphism. Elevation of CPK led us to suspect the diagnosis of DMD. NBT test and phagocytic functions studies confirmed a chronic granulomatous disease, suggesting therefore a large Xp21 deletion. QMPSF (Quantitative Multiplex PCR of Short Fluorescent Fragments) was performed to identify and characterize the deletion in both cases. In patient 1, a 5 Mb deletion removed exons 53-79 of DMD and the entire GK, DAX1 / NROB1 and IL1RAPL1 genes. In patient 2, a 7 Mb deletion encompassed exons 1-52 of DMD and the entire XK and CYBB genes. In both cases, QMPSF easily allowed the determination of the mother's carrier status and therefore facilitated genetic counseling. This report shows that QMPSF can be efficiently used to detect and map contiguous gene deletions.

**Ring 15 Syndrome: Clinical, Cytogenetic and Comparative Genomic Hybridization-Array Characterization of Two Discordant De Novo Cases.** *I.A. Glass<sup>1</sup>, E. Chen<sup>2</sup>, K.A. Rauen<sup>2</sup>, D.G. Albertson<sup>2</sup>, D. Pinkel<sup>2</sup>, P.D. Cotter<sup>2</sup>.* 1) University of Washington, Seattle, WA, USA; 2) University of California San Francisco, CA, USA.

Ring chromosome 15 is an unusual finding with less than 50 patients reported. Despite this rarity a wide range of outcomes resultant from the ring is evident, ranging from a near-normal outcome to prenatally recognizable birth defects. However, precise genotype-phenotype correlations are problematic because of the difficulties determining the extent of euchromatic loss, the extent of mosaicism, and the timing of ascertainment/characterization coupled with the potential loss of the ring during mitosis. We report, two discordant examples of a chromosome ring 15. In the first case, prenatal diagnosis of a case of a de novo ring 15 manifesting IUGR, single umbilical artery and a thickened nuchal fold was made, following amniocentesis at mid-trimester: 46,XX,r(15)(p13q26). Post-natal follow-up confirmed growth retardation, mild microcephaly, triangular facies, cafe au lait macules, brachydactyly, congenital heart disease, neonatal seizures, microphthalmia and developmental delay. The second patient, a 31 year old female was karyotyped because of her short stature and dilated cardiomyopathy; 46,XX,r(15)(p11q26). Although, mild intellectual disability (requiring special education) was present she was living and working independently. There was disproportionate short stature (-4 HT-SDS;US:LS = 0.91), small hands and feet and a unilateral blue iris heterchromia present. To investigate the discordance in phenotypes between the two patients, we undertook further molecular cytogenetic evaluations of both cases, including FISH and array CGH analyses in order to more fully characterize the extent of the deletions associated with these r(15) chromosomes. Case 1, manifesting the more severe phenotype showed deletion of multiple clones, consistent with approximately a 6Mb deletion of distal 15q. By contrast, case 2 with the milder phenotype, only revealed loss of the 15q subtelomere clone by array CGH.

**High resolution CGH in the characterization of cryptic and non-cryptic chromosome imbalances.** *X.L. Huang<sup>1</sup>, H.E. Wyandt<sup>1,2</sup>, J.M. Milunsky<sup>1,3,4</sup>*. 1) Center for Human Genetics, Boston Univ Sch Medicine, Boston, MA; 2) Dept of Pathology; 3) Dept of Pediatrics; 4) Dept of Genetics and Genomics.

High-resolution comparative genomic hybridization (HRCGH), using Cytovision Hi-resolution CGH software, Version 2.77 (Applied Imaging, Santa Clara, CA), was done to further characterize several structural chromosome abnormalities previously identified by conventional cytogenetics (CC) and/or FISH. These included: 1) dup(3)(p?21p?25)de novo; 2) rec(2)dup(2q)inv(2)(p25.3q32.2)mat; 3) add(11)(p15)mat; 4) add(X).ish der(X)t(X;15)(q?26;q?24)de novo; 5) +der(9)t(8;9)(p23;q?13)mat; 6) r(22)(p11.1q13)de novo; 7) ish del(9)(q34.3)de novo; 8) ish +i(18)(p10)de novo; 9) ish +r(2)(p?12q?12)de novo. The above abnormalities, except for case 9, were confirmed by HRCGH. The +r(2), with euchromatin identified by chromosome painting, was present in only 55% of cells. This imbalance most likely was not detected by HRCGH, due to the mosaicism. Generally, imbalances of segments in or near centromeres and the ends of chromosome arms (e.g. cases 5,7 and 8) were not detected, although they were clearly involved. In cases 2-4, breakpoints obtained by HRCGH revised or clarified those estimated by CC. In case 2 with rec(2), detectable loss in the terminal band of 2p extended more proximal by HRCGH than was determined by CC. Similarly, breakpoints in 11p (case 3) and Xq (case 4) were revised to more proximal bands by HRCGH. An extra copy of distal 15q in der(X)t(X;15) (case 4) was not identified by CC, but was readily detected by HRCGH. In case 5, a small piece of distal 8q on der(9)t(8;9)mat was not initially identified by CC, but was detected by HRCGH. A cryptic del(9q) detected by subtelomeric FISH (case 7) was confirmed by HRCGH. Regions in or near centromeres and telomeres, dosages of segments <3Mb, or mosaicism <60% are reportedly not reliably detected by HRCGH. Nevertheless, three of our cases (3, 5, and 7) with deletion or duplication in terminal bands were detected by HRCGH. The benefits and limitations of HRCGH as an alternative to other FISH techniques for identifying euchromatic imbalances are demonstrated.

**Subtelomeric FISH Analysis in 108 Autistic Patients as Adjunct to Chromosome Analysis and Fragile X Testing.**

*L. Medne, K. Russell, J. Ming, I.D. Krantz, M. Souders, S. Levy, A. Gupta, N.B. Spinner, E.H. Zackai, J.J.D. Morrissette.* Div Hum Gen & Molec Biol, Clin, The Children's Hospital of Philadelphia, Philadelphia, PA.

The recent development of molecular probes that allow for detection of subtelomeric chromosomal rearrangements by FISH has led to the emergence of submicroscopic telomeric rearrangements as an important cause of human malformations and neurodevelopmental disabilities. Autistic spectrum disorder (ASD) represents a broad range of cognitive and neurobehavioral impairments that cause qualitative deficits in socialization, communication and behavior. ASD can be associated with various genetic syndromes and chromosomal abnormalities. In the vast majority of patients, ASD is non-syndromic without an identifiable genetic etiology. In addition to standard karyotyping and Fragile X testing, we performed subtelomeric FISH analysis in 108 patients with ASD. A clinically recognizable syndrome was ruled out. 8/108 (7.4%) had a chromosome rearrangement, only one of which was an unbalanced subtelomeric translocation not detectable cytogenetically. Of the seven cytogenetically detectable chromosomal rearrangements, two had an unbalanced translocation: 46,XX, der(8) t(4;8)(q34;q32); 46,XY, der(4) t(4;4)(p12q28); one had a terminal deletion 46,XY, del(21)(q22.1); three patients had cytogenetically balanced rearrangements and their clinical significance was unknown as they were inherited from an unaffected parent: 46,XY, t(3;4)(q25;q27) mat, 45,XY, t(13;14)(q10;q10) pat and 46,XX, inv(7)(q22.1q34) mat. The last patient had 46,XY, 15p+ (ish.subtel x 2) and the clinical correlation is also not known. The patient with the subtelomeric unbalanced subtelomeric translocation had 46,XY.ish der(18) t(13;18)(q34;q23). Based on the findings of this study, the utility of subtelomeric FISH analysis remains to be further clarified.

**Utilization of FISH analysis to identify mosaicism in Ullrich-Turner syndrome patients.** *A. Wiktor, D.L. Van Dyke.*  
Medical Genetics, Henry Ford Health System, Detroit, MI.

Ullrich-Turner syndrome (UTS) is most commonly associated with a 45,X karyotype, but is seen in patients with a variety of X-chromosome abnormalities or 45,X/46,XY mosaicism. The phenotype of UTS patients is highly variable, and depends largely on the karyotype. Patients are at an increased risk of gonadoblastoma when a Y-derived chromosome is present. Since constitutional mosaicism is present in approximately 50% of UTS patients, the identification of minor cell populations is clinically important and a challenge to laboratories. We reviewed our cytogenetic data on 55 females with a 45,X karyotype as the sole abnormality or as part of a more complex karyotype. Seventeen (31%) revealed a 45,X karyotype. There was X/XX/XXX or X/XXX mosaicism in 20 (36%) samples, four (7%) patients had an iso(Xq), two (4%) had deletions of the X chromosome (Xq21.1 and Xp11.2), and five (9%) had a ring X chromosome. Mosaicism with a structurally normal Y chromosome was seen in three (5%) cases, two (4%) patients had a dicentric Y, and one (2%) case had a small marker that was determined to be derived from the Y. One (2%) patient showed mosaicism for a marker chromosome that was derived from neither the X nor Y chromosomes. To further investigate the possibility of Y chromosome material in the 17 patients with an apparently non-mosaic 45,X karyotype, we performed FISH using centromere probes for the X and Y chromosomes. A 45,X result was confirmed in 11 cases, and a minor XX cell line was identified in 6 patients. Two of the six samples were found to have a second structurally abnormal X chromosome when metaphase cells were analyzed by FISH or G-banded slides were re-examined. One patient had an i(X)(q10) and the other had an idic(X)(q25). No samples with a XY mosaicism were identified. While several publications have documented the use of PCR-based assays to identify mosaicism in UTS patients, the incidence of false positives using PCR testing has raised concern with these assays. We believe the use of FISH as an adjunct to karyotype analysis provides a sensitive and cost-effective technique to identify sex chromosome mosaicism in UTS patients with a 45,X karyotype.

**Use of telomere and specific locus FISH probes to detect deletions within duplications.** *J. Panella<sup>1</sup>, M. Williams<sup>4</sup>, C. Miller<sup>1</sup>, J. Johnson<sup>1</sup>, W. Wells<sup>1</sup>, Z. Qi<sup>2</sup>, L. Meisner<sup>1,3</sup>, J. Yu<sup>1,2,3</sup>.* 1) Wisconsin State Laboratory of Hygiene; 2) The Waisman Center; 3) Department of Pathology and Lab Medicine, University of Wisconsin-Madison, Madison, WI; 4) Gunderson Lutheran Hospital, La Crosse, WI.

We present an 18 month old child who is the product of an uncomplicated pregnancy with a non-contributory family history. Concerns relating to delayed development were raised at 3 months, and by 17 months neurological testing showed that both gross and fine motor skills were at the 10-11 month level. Physical examination demonstrated mild to moderate facial dysmorphic features but a normal neurological exam except for the developmental delay. MRI of the brain showed a thin anterior corpus callosum and apparently absent splenium, with focal areas of abnormal high signal in the periventricular white matter. High resolution cytogenetic analysis with G-banding demonstrated an extra dark band in the 8p23 region that was similar in appearance to band 8p22, suggesting a duplication of the 8p22-23 region. Since both parents were cytogenetically normal, we used a chromosome 8 painting probe with fluorescence in situ hybridization (FISH) to establish that the duplicated material derived from chromosome 8. We then microdissected the 8p23.1 band, and made a probe for this region. Since this probe yielded two signals of different sizes, we used an 8p sub-telomere probe to better evaluate the distance between the telomere and the duplicated region. The telomere and specific locus FISH signals, combined with inverted DAPI banding, suggested an inverted duplication of part of 8p21, all of p22, and part of 23.1. In addition, the 8p terminal region distal to the breakpoint within 8p23.1 was deleted. Therefore, the karyotype of the patient is 46,XX,der(8)del(8)(p23.1)dup(8)(p23.1p21). The FISH studies enabled obtaining more detailed genetic information for the clinical assessment of the patient, and for genotype/phenotype association studies. This study also demonstrated the necessity of using molecular cytogenetic techniques to more precisely analyze de novo chromosome aberrations that cannot be interpreted by standard prometaphase banding alone.

**A Case of Twin Pregnancy: a Coexisting of 46, XY Heterozygous Complete mole; and a Normal 46, XX Fetus.** *O. Samura, N. Miharu, Y. Nakashima, N. Fujito, M. Hyodo, N. Honda, K. Ohama.* Dept OB/GYN, Hiroshima Univ Sch Medicine, Hiroshima, Japan.

Hydatidiform moles with a coexisting live fetus are rare; the incidence is estimated at from 1:22000 to 1:100,000 pregnancies. Three variants of the condition are known: (1) twin pregnancy with a complete hydatidiform mole and coexistent normal fetus (CMCF); (2) twin pregnancy with a partial hydatidiform mole and coexistent normal fetus; and (3) a partial hydatidiform mole. Of the three variants, CMCF has the highest potential for developing persistent trophoblastic disease (PTD); thus, it must be differentiated from the other types. Especially CMCF with the 46,XY heterozygous mole is very rare, only three cases have been reported. We report the case which had a normal infant without developing persistent trophoblastic disease (PTD). A 30-year-old pregnant woman delivered a normal female infant and the molar tissue at 28 weeks of gestation. Cytogenetic analyses of the mole and fetus were performed. We analyzed the genetic origin by quantitative fluorescent PCR (QF-PCR) with short tandem repeat markers. Chromosomal analysis revealed that the karyotype of the molar tissue was 46, XY, and the fetus was 46, XX. The molar tissue showed a 46,XY heterozygous complete mole using QF-PCR. This case indicates that a patient can achieve a good pregnancy outcome even if the pregnancy consists of CMCF with a 46, XY heterozygous mole. In addition, QF-PCR is useful method for the determination of the genetic origin of the mole and the infant.

**Delineation of the origin and structure of chromosomal duplications: analysis of 136 cases.** *R. Tarvin, L. Christ, C. Curtis, S. Schwartz.* Case Western Reserve University and University Hospitals of Cleveland.

The routine use of FISH analysis in the cytogenetic laboratory has identified chromosomal duplications arising from either duplication of material from the same chromosome (intrachromosomal) or from another chromosome (interchromosomal). They may be a simple duplication of material or they may involve the loss or rearrangement of other material. In order to better understand the structure, origin and nature of duplications, we have undertaken a systematic study of 136 duplications, which were not due to the malsegregation of parental rearrangements. These have been analyzed using high-resolution chromosome analysis, FISH with chromosomal libraries, in many cases, analysis with microsatellite markers and lastly, employing data from the Human Genome Project, the utilization of over 250 BACs to fully characterize many of the duplications.

This is the most comprehensive study of duplications to date and results from this work have lead to a number of findings regarding this abnormality. (1) The majority of duplications are intrachromosomal although 39% are interchromosomal; (2) the structure of these abnormalities is easily determined by FISH and in 94% of the cases only one library needed to be used to delineate the chromosomal origin of the extra material; (3) chromosomes 8, 9, 15, 18 and X are preferentially involved; (4) the abnormalities can originate in either paternal (37%) or maternal meiosis (67%); (5) overall 93% were de novo duplications, but in 7% of the cases a parent had the same duplication; the majority of these were intrachromosomal; (6) approximately 14% of these were ascertained serendipitously without an associated phenotype; (7) in 29% of the cases the structure was more complex than just that of a simple duplication; most of these involved a deletion in addition to the duplication; (8) approximately 20% of these duplications were associated with a specific syndrome and in 5% of the cases a duplication of a specific gene was diagnostic; and (9) in 10% of the cases cryptic telomeric duplications, most, which did not involve the subtelomere, were delineated by this work.

**Constitutional abnormalities of chromosome 20: Report of 2 rare new cases and review.** *J. Garcia-Heras, R.A. Kilani, K. Grange, R. Martin, S. Lamp, J. Ivanovich.* Dept Pediatrics, Washington Univ Sch of Med, St Louis, MO.

We report 2 unbalanced rearrangements of chromosome 20 in newborns and review the literature to establish genotype-phenotype correlations. Case A is a male born at 37 wks by vaginal delivery to a 33-year-old G5P2 mother. The APGAR score was 2, 6 and 7 at 1, 5 and 10 minutes respectively. The birth weight was 2,400 GM (40th centile), the length was 47 cm (25th centile) and the OFC was 32 cm (25th centile). The child had craniofacial dysmorphism, normal male genitalia, a small phallus with a semibifid scrotum, and bilateral widely separated 1st and 2nd toes, A hypoglycemia since birth and the small phallus were due to panhypopituitarism. GTW banding in lymphocytes detected an interstitial deletion of band 20p11.2. The paternal karyotype was normal. The mother carried in lymphocytes the same deletion of 20p11.2 in mosaicism so this case would be the 2nd deletion of 20p inherited from a mosaic carrier parent. Our case and a deletion of 20p11.22-p11.23 with autism, minor facial anomalies and Hirschprung disease suggest that monosomies of 20p11.2 have a mildly affected phenotype. Case B is a female born at 40 wks by C-section to a G1P1 27-year-old mother. At birth the weight was 3,507 GM (75th centile), the length was 51.5 cm (75th-90th centile), and the OFC was 33 cm (25th centile). The APGAR score was 6 at 1' and 7 at 5'. The child had craniofacial dysmorphism, bilateral 5th finger clinodactyly, broad thumbs and mildly broad great toes. An echocardiogram showed a complex cardiac defect. Multiple thoracic vertebral anomalies, 12 pairs of ribs and 6 lumbar vertebrae were seen by a chest X-ray. GTW banding in lymphocytes demonstrated a rec(20) dup 20q with a deletion of 20p13->pter and a trisomy of 20q13.1->qter. The maternal karyotype was normal. The rec(20) was inherited from the father carrier of an inv(20)(p12q13.11). This first report of a rec(20) dup 20q and 3 other trisomies 20q13->qter have a normal intrauterine growth, a congenital cardiac defect, craniofacial dysmorphism and hand, feet, rib and spine anomalies. Subtelomeric deletions in the chromosomes involved in the translocations or the 20p deletion in the rec(20) explain their clinical differences.

**Cleft palate, polydactyly and mild mental retardation in a girl with a balanced chromosome 7;9 translocation.** *P. Gustavsson, E. Gourgouvelis, E. Blennow, A. Ilicki, M. Nordenskjöld.* Department of Molecular Medicine, Karolinska Hospital, Stockholm, Sweden.

We have identified a girl carrying a chromosomal translocation associated with cleft palate, polydactyly and learning difficulties. The girl is the only child of healthy parents. Chromosome banding revealed an apparently balanced translocation involving chromosomes 7p and 9p. The karyotype is defined as 46,XX,t(7;9)(p21;p21).

Previous studies have shown that patients carrying mutations in the TWIST gene located on chromosome 7p21 present with Saethre-Chotzen syndrome characterized by multiple anomalies such as facial malformations, craniosynostosis, hand and foot abnormalities. Also, microdeletions spanning the TWIST locus are associated with mild mental retardation suggesting a contiguous gene syndrome.

We have used fluorescent in situ hybridization (FISH) to metaphase chromosomes in order to map the translocation breakpoints on chromosome 7p and 9p. Our results revealed that no known gene was disrupted by the chromosome 9p breakpoint. FISH analysis show that the chromosome 7p breakpoint is located 3' of the TWIST gene. In addition, the breakpoint on chromosome 7p is located close to a gene encoding histone deacetylase 9 (HDAC9). HDAC9 is predominantly expressed in the brain and the gene product is involved in histone deacetylation that regulates gene expression.

We suggest that the chromosome 7p breakpoint is involved in the aetiology of malformations and learning difficulties in the patient. The mechanism might be explained by a positional effect on adjacent genes caused by the translocation breakpoint. Further FISH and molecular analysis is in progress in order to refine the chromosome 7p breakpoint in relation to known and unknown genes.

**22q11.2 deletions in familial DiGeorge/Velocardiofacial syndrome are predominantly smaller than the commonly observed 3Mb.** *A. Adeyinka<sup>1</sup>, K.J. Stockero<sup>1</sup>, H.C. Flynn<sup>1</sup>, L.K. Courteau<sup>2</sup>, C.P. Lorentz<sup>1</sup>, R.P. Ketterling<sup>1</sup>, D.B. Dawson<sup>2</sup>, S.M. Jalal<sup>1</sup>.* 1) Cytogenetics Laboratory, Pathology and Laboratory Med, Mayo Clinic, Rochester, MN; 2) Molecular Genetics Laboratory, Pathology and Laboratory Med, Mayo Clinic, Rochester, MN.

DiGeorge/Velocardiofacial syndrome (DG/VCFS), cytogenetically characterized by deletion of 22q11.2, is the most common microdeletion syndrome with an incidence of about 1: 4000. In over 88% of patients, the deletion size is 3 Mb while the remaining have deletions between 1.5 Mb and 2.5 Mb. Recently, we described a number of patients with duplication of 22q11.2 which is the reciprocal recombinant product of the 22q11.2 deletion. The duplication sizes observed ranged from 3 Mb to 6 Mb, and included a family of 3 affected siblings who inherited a 4 Mb duplication from their mother. Interestingly, of approximately 250 well-characterized microdeletion cases in the literature, no deletion sizes over 3 Mb was reported. This observation supports the notion that large duplications of the genome are better tolerated than large deletions and has led us to hypothesize that small deletions may be tolerated in a familial fashion more easily than large deletions. To test this hypothesis, FISH probes designed from bacterial artificial chromosomes (BACs) and P1 artificial chromosomes (PACs) mapped to 22q11.2 were used to determine the deletion sizes in 13 individuals from six families with cytogenetically proven 22q11.2 deletion. Three families (7 individuals) had deletions of less than 3 Mb (1.5 Mb to 2 Mb) and 3 families (6 individuals) had 3 Mb deletions. Molecular analysis based on a panel of 15 STR markers is underway to confirm these findings. The 50% frequency of smaller deletions among this group of patients with familial del(22)(q11.2) is significantly higher than that reported (upper limit of 22%) in the general population. These preliminary findings support the hypothesis that smaller sized del(22)(q11.2) are more common in a familial setting and underscores the need to exclude a familial basis in all cases of del(22)(q11.2) smaller than 3 Mb.

**Segregation of a novel euchromatic expansion of 8p22 in three generations with no associated phenotypic abnormalities.** *S. Chan*<sup>1</sup>, *F.J. Dill*<sup>1,2</sup>, *S. Langlois*<sup>1,2</sup>, *J.T. Pantzar*<sup>1</sup>, *B. Lomax*<sup>1</sup>, *E. Rajcan-Separovic*<sup>1</sup>. 1) Pathology and Laboratory Medicine; 2) Medical Genetics, Children's and Women's Health Centre of British Columbia, University of British Columbia, Vancouver, Canada.

Rearrangements of the short arm of chromosome 8 encompass a wide variety of cytogenetic abnormalities, many of which are recurrent. Recent studies have suggested that the presence of two olfactory receptor gene clusters in 8p may be involved in the generation of some of these rearrangements. We present the first report of a euchromatic expansion of 8p22 which segregates in three generations of a family with no associated phenotypic abnormalities.

A 30 year-old, G<sub>2</sub>P<sub>1</sub> woman presented with an increased risk of Down syndrome on triple maternal serum screen. Amniocentesis was performed and cytogenetic studies on G-banding preparations showed an expansion of the G-positive band 8p22 in all cells examined. FISH with chromosome 8 paint indicated that the extra genetic material was chromosome 8 in origin. CGH using DNA extracted from the cultured amniocytes revealed a small gain in the distal portion of 8p22. Investigations on the family members showed identical findings in the phenotypically normal mother and maternal grandfather. C-banding performed on maternal blood lymphocytes revealed no C-band positive material in the 8p22 region, providing evidence that the extra genetic material was euchromatin. Given the results, the couple was counseled that this cytogenetic finding was a familial variant unlikely to be associated with an increased risk of developmental anomalies. Despite this, the family elected to terminate the pregnancy. Autopsy revealed no fetal anomalies.

As 8p22 is a G-positive band and likely to be gene-poor, this may explain the absence of phenotypic abnormalities in association with the cytogenetic finding observed in this family. Given that the location of 8p22 expansion in this case is in close proximity to that of an olfactory receptor gene cluster reported in the literature, it likely represents a rearrangement originating from or in association with these repetitive sequences, the exact nature of which is to be determined by further studies.

**Infant with maternal UPD14 heterodisomy arising through a de novo dic(14:14)(p12;p12) formed during maternal meiosis I.** *C. Curtis<sup>1</sup>, M. Falk<sup>1</sup>, A. Zinn<sup>1</sup>, N. Bass<sup>2</sup>, S. Schwartz<sup>1</sup>.* 1) Center for Human Genetics; 2) Dept Pediatric Neurology, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, OH.

A 9-month-old infant was referred for genetic evaluation of his hypotonia, hypoactive reflexes and small size. Testing provided no evidence of a metabolic disorder or Prader-Willi syndrome. Cytogenetic analysis, however, revealed a male karyotype with 45 chromosomes including a dicentric chromosome composed of two long arms of chromosome 14 [45,XY,dic(14;14)(p12;p12)]. Parental karyotypes were normal. Molecular analysis of chromosome 14 DNA markers revealed a pattern of inheritance in the patient consistent with maternal disomy, without inheritance of any paternal alleles at informative loci. Most interestingly, DNA marker D14S990, located in G-band region 14q11.2 and the most proximal marker tested, exhibited maternal heterodisomy. This suggests that both maternal chromosome 14 homologues participated in the formation of the de novo dic(14;14). D14S48, a more distal marker in 14q31-q32, exhibited maternal isodisomy. This study revealed that: (1) Because maternal heterodisomy was present in the most proximal markers analyzed while maternal isodisomy was present in distal markers, the dicentric most likely formed between non-sister chromatids after completion of crossing-over. (2) The infant's UPD14mat most likely resulted from post-zygotic trisomy rescue of a 46,XY,+14,dic(14;14)(p12;p12) conceptus through loss of the normal paternal chromosome 14, though such a 46 chromosome cell-line was not detected in the patient's peripheral blood. (3) The infant's phenotype of small size and hypotonia correlates with previously reported cases of i(14q) isodisomy and normal karyotypes (46,XX or 46,XY) with UPD14 heterodisomy. (4) UPD14 testing should be considered in infants with hypotonia, normal methylation of 15q11.2-15q12 and no evidence of a metabolic disorder, since the remaining common features of UPD14mat (precocious puberty, short stature, developmental delay and/or mental retardation) may not become evident until some time later.

**Prenatal cytogenetic detection of an unbalanced karyotype involving 9p- and 18q duplication in a fetus with apparently normal phenotype.** *C.C. Lin<sup>1,3</sup>, L.J. Hsieh<sup>1</sup>, Y.J. Pan<sup>2</sup>, Y.C. Li<sup>3</sup>.* 1) Department of Medical Research, China Medical University Hospital, Taichung, Taiwan; 2) Department of Obstetrics and Gynecology, Lin Shin Hospital, Taichung, Taiwan; 3) Department of Life Sciences, Chung Shan Medical University, Taichung, Taiwan.

A 26-year-old woman was referred for prenatal diagnosis of chromosomal disorders at 17 weeks gestation due to a maternal serum triple screen resulting in a Down syndrome risk estimate of 1 in 162. A fetus sonogram did not show any structural abnormality. The pregnancy was uneventful up to the time the test was performed. G-banding and SKY analyses revealed the fetus had a female sex with a derivative chromosome 9 that was resulted from a translocation of the chromosome 18 segment distal to 18q11.2 to the short arm of chromosome 9 at band 9p22. In addition, FISH study showed the presence of a chromosome 18q sub-telomeric sequence in the short arm of the derivative chromosome, thus verified the translocated segment containing 18qter. Chromosome studies on the parents of the fetus were also performed and found both parents have normal chromosome complement. Therefore, the fetus was identified of having an unbalanced karyotype, 46,XX,der(9)t(9;18) (p22;q11.2) de novo. The pregnancy was terminated at 21 weeks and other than the slightly low-set ears, the fetus appeared as having a normal phenotype. Follow up chromosome studies on a cord blood sample and a skin sample confirmed early cytogenetic findings. To our knowledge, this is the first reported case of an apparent normal fetus with an unbalanced karyotype involving 9p- syndrome and 18q duplication syndrome. The study was supported by grants from National Health Research Institute (NHRI-EX92-9207 SI) and National Science Council (NSC 91-2320-B-040-037).

**Terminal deletions of 4q in phenotypically normal individuals.** *J.T. Mascarello<sup>1</sup>, B.R. Ravnan<sup>1</sup>, C.L. Martin<sup>2</sup>, M. Verp<sup>2</sup>, E. Weber<sup>2</sup>.* 1) Genetics, Santa Fe, NM; 2) University of Chicago, Chicago IL.

We report five families with at least one phenotypically normal carrier of a terminal deletion of the chromosome 4 long arm. In four families, the deletion involved the microscopically-detectable loss of chromatin from band q35. In all four cases, the terminal nature of the deletion was demonstrable by subtelomere FISH analysis. In the fifth, the deletion was detected by subtelomere FISH. Results of G-band analyses in this family were not available but, presumably, would have shown no deletion because the deletion removed the Vysis subtelomere target but not the CytoCell subtelomere target. These cases, in conjunction with previous reports of satellited chromosome 4 long arms demonstrate that the loss of distal 4q often produces no clinical consequences. Moreover, such cases point to a need for caution in interpreting results of subtelomere studies that show a deletion of terminal 4q.

**Infant with homozygous premature chromatid separation trait: Autopsy findings of bilateral Wilms tumors and rhabdomyosarcoma of the bladder.** *H. Numabe*<sup>1, 2</sup>, *T. Ikeuchi*<sup>3</sup>, *T. Kajii*<sup>4</sup>, *M. Tachibana*<sup>2</sup>, *K. Iso*<sup>5</sup>, *E. Arai*<sup>6</sup>, *T. Ishida*<sup>6</sup>, *K. Mukai*<sup>6</sup>. 1) Dpt of Medical Informatics, Tokyo Medical Univ, Tokyo, Japan; 2) Dpt of Pediatrics, Tokyo Medical Univ Hosp, Tokyo, Japan; 3) Dpt of Molecular Cytogenetics, Medical Research Institute, Tokyo Medical and Dental Univ, Tokyo, Japan; 4) Hachioji, Tokyo, Japan; 5) Dpt of Obstetrics and Gynecology, Tokyo Medical Univ Hosp, Tokyo, Japan; 6) First Dpt of Pathology, Tokyo Medical Univ, Tokyo, Japan.

We report a girl with homozygous premature chromatid separation (PCS) trait, chromosomally characterized by PCS and mosaic variegated aneuploidy (MVA). She had pre- and postnatal growth retardation, microcephaly, Dandy-Walker malformation, and minor facial abnormalities; and at age 22 weeks developed generalized seizures controllable with phenobarbital. At age 4 months, bilateral multicystic Wilms tumors and rhabdomyosarcoma of the bladder were detected. She died of renal failure at age 9 months. Autopsy findings will be described. All tissues and cells from the infant analyzed showed a high frequency of PCS and MVS. They included amniotic fluid cells at 34 weeks of gestation; placental chorionic villous cells and cord blood lymphocytes at delivery; neonatal peripheral blood lymphocytes; and lung fibroblasts, kidney fibroblasts, and Wilms tumor cells obtained on autopsy.

**Molecular characterization of a (14)(q23q24) deletion in a patient with features of Holt-Oram syndrome. C.**

*Michel-Adde<sup>1</sup>, N. Le Meur<sup>1</sup>, A. Goldenberg<sup>1</sup>, V. Drouin-Garraud<sup>1</sup>, G. Blaysat<sup>2</sup>, S. Marret<sup>2</sup>, S. Abu Amara<sup>3</sup>, H. Moiro<sup>1</sup>, P. Saugier-veber<sup>1</sup>, T. Frebourg<sup>1</sup>, A. Rossi<sup>4</sup>.* 1) Department of Genetics, Rouen University Hospital, France; 2) Department of Paediatrics, Rouen University Hospital, France; 3) Department of Paediatric Surgery, Rouen University Hospital, France; 4) Department of Genetics, EFS-Normandie, Bois-Guillaume, France.

Holt-Oram syndrome, the major "hand-heart" syndrome, is defined by the association of radial defects or triphalangeal thumb and atrial septal defect. The transmission is autosomal dominant and the causative gene has been shown to be TBX5 located on 12q24.1 which encodes a transcription factor. The genetic heterogeneity of this syndrome has been suggested by several reports. We report here on a 14q23q24 deletion in a boy presenting with severe bilateral asymmetrical radial aplasia, congenital heart defect and poor neurological outcome. This deletion was shown to be inherited from the mother who carried a balanced interchromosomal insertion (46, XX, ins(4;14)(q31.1;q23q24)). This is the second report a 14q23q24 deletion associated with features of Holt-Oram syndrome. In our case, molecular cytogenetic analysis demonstrated that the deleted 14q material encompassed several genes encoding transcription factors potentially involved in heart and bone development. This observation strongly suggests the implication of a new "heart-hand" locus on chromosome 14q.

Program Nr: 861 from 2003 ASHG Annual Meeting

**Molecular characterization of an atypical 5p microdeletion in a child with dysmorphic features and developmental delay.** *M.D. Speevak, D. Cushing, M. Wood, S.A. Farrell.* Dept Genetics, Credit Valley Hosp, Mississauga, ON, Canada.

Cri du chat syndrome is a contiguous gene disorder associated with a partial short arm deletion of chromosome 5 and typical clinical features including a cat-like cry, distinct facies and severe global delay. The relevant breakpoint begins at 5p15 and extends through to the short arm terminus. We report a case of a four year old boy with a small interstitial deletion detected by G-banding, at band 5p15.2; however, FISH using the Cri du chat probe (Vysis) failed to confirm the deletion. The child had mild developmental delay and subtle dysmorphic features with a small lower jaw and slightly prominent ears but none typical of Cri du chat syndrome. Because the clinical features and FISH analysis were not consistent with a diagnosis of Cri du chat syndrome, chromosome band walking was performed using fluorescent probes generated from BAC clones labeled by degenerate oligomer primed (DOP) PCR. An atypical deletion involving bands 5p14.2 and 5p15.1 was revealed. These results demonstrate that unique custom probes are valuable tools for the confirmation of suspected rearrangements when commercial probes are uninformative.

Program Nr: 862 from 2003 ASHG Annual Meeting

**Prenatal diagnosis of a fetus with trisomy for the 16p subtelomere.** *G. Sekhon, N. Agan, J. Branson, A. Salvador.*  
Cytogenetics Lab, St John's Mercy Medical Ctr, St Louis, MO.

Routine analysis for abnormal ultrasound led to the prenatal diagnosis of a fetus with additional euchromatic material on the short arm of chromosome 20. Ultrasound on a 24 week fetus showed the presence of multicystic left kidney and right kidney hydronephrosis. Additional material on the short arm of chromosome 20 and multicystic kidney disease prompted us to use the whole arm painting probe for chromosome 16. This and further FISH mapping studies with probes from the polycystic kidney gene PKD1 confirmed our suspicion that the extra material is from chromosome 16 ; hence, the cytogenetic diagnosis was given as 46,XX,der(20)t(16;20)(p13.2;p13).ish der(20) (D16S3400x3,D20S1157x2). The breakpoint on the short arm of chromosome 20 occurred distal to the short arm specific telomere probe and did not result in any monosomy of chromosome 20. Parental chromosomes were normal. Only by supplementing conventional cytogenetic methods with molecular cytogenetic techniques could the true karyotype be unequivocally determined.

**Cytogenetic mapping of PKD2 gene to 4q21 within the critical region for the 4q- macrocephaly phenotype.** *M. Velinov*<sup>1,2</sup>, *M.J. Macera*<sup>3</sup>, *J. Kupferman*<sup>2</sup>, *H. Gu*<sup>1</sup>, *A. Babu*<sup>3</sup>, *E.C. Jenkins*<sup>1</sup>, *G. Kupchik*<sup>2</sup>. 1) Dept Cytogenetics, IBRDD, Staten Island, NY; 2) Maimonides Medical Center, Brooklyn, NY; 3) Wyckoff Heights Medical Center, Brooklyn.

A 3-year-old boy presented with growth and developmental delay, hypotonia, relative macrocephaly, dysmorphic features and polycystic kidneys. He had a small interstitial deletion of chromosome 4q (karyotype 46,XY,del (4)(q21.1q21.3). This patient's phenotypic findings were consistent with the previously reported deletion 4q21/4q22 macrocephaly phenotype (Nowaczyk M.J.M et al., Am J Med Genet 68:400-405, 1997). Previous linkage studies have localized the gene for type II polycystic kidney disease, PKD2, to bands 4q21-q23. FISH analysis, with a BAC probe containing the PKD2 gene, demonstrated hemizyosity for this locus in our patient since no signal was detected over the del (4) chromosome. This provided a more precise cytogenetic localization for PKD2. Cytogenetic findings in our patient appear to limit the deletion to 4q21.1-4q21.3. Band 4q22 was cytogenetically present. The reported deletion also better defines the minimal critical region for the 4q- macrocephaly phenotype. The absence of one of the PKD2 alleles in our patient had likely contributed to the early bilateral development of renal cysts. For comparison, previously reported patients with non-PKD1 (most likely PKD2) mutations had ultrasound - identifiable renal cysts before the age of 30 in only 11% of cases. Thus in our patient PKD2 hemizyosity is associated with a more severe variant of type II polycystic kidney disease. *This work was supported in part by the new York State Office of Mental Retardation and Developmental Disabilities.*

**Understanding placental mosaicism: two unusual cases of apparently confined placental mosaicism found in the neonate.** *G. Stetten, C.S. Escallon, J.L. McMichael, D.O. Saul, K.J. Blakemore.* Johns Hopkins University, Baltimore, MD.

Confined placental mosaicism (CPM) is a tissue specific mosaicism, often involving an aneuploid cell line identified in the placenta and apparently absent from the fetus. When mosaicism is identified through chorionic villus sampling (CVS) patients are counseled about options including follow-up ultrasound, amniotic fluid (AF) analysis, fetal blood and/or skin biopsy as well as possible uniparental disomy (UPD) studies. If these studies are normal, then it is highly likely that the fetus has a normal, non-mosaic karyotype. In 4000 CVS we have identified placental mosaicism in 38 cases. In 29 of these a normal karyotype was obtained from AF. In 28 the pregnancy progressed to a live birth with a normal phenotype. In 18 cases cord blood was karyotyped and found to be normal. UPD testing was offered as indicated, and in four cases showed normal, biparental inheritance of the given chromosome. In two of the 28 cases the abnormal karyotype thought to be confined to the placenta was found in the baby. In one case the CVS showed three cell lines 48,XX,+18,+mar[19]/47,XX,+18[12]/46,XX[5]. The AF was 46,XX in 33 colonies. However, cord blood at birth showed 3/100 cells with the trisomy 18 karyotype, 47,XX,+18[3]/46,XX[97]. In the second case an accessory derivative chromosome 10 was seen in the CVS, 47,XY,+der(10)[15]. Because the cytogenetic abnormality was inconsistent with a normally developing fetus, an amniocentesis was recommended. The AF was 46,XY in 80 colonies. At birth cord blood showed 20/20 cells with the normal karyotype. Mosaicism was confirmed in the placenta as expected, 47,XY,+der(10)[33]/46,XY[4], but surprisingly found in the skin as well, 47,XY,+der(10)[3]/46,XY[16]. The difficulties encountered when what was thought to be CPM is found in a neonate present a new diagnostic dilemma. We recommend follow-up at birth because it is important to know the degree of mosaicism in the population and its association with both normal and abnormal phenotype. However, the information must be presented to patients with great care, since a low level, mosaic chromosomal abnormality may or may not be benign.

**Development of Genomic Resources and Cytogenetic Databases for Patient Sample Characterization and Disease Gene Discovery.** *J.S. Herbrick<sup>1</sup>, E. Kanematsu<sup>1</sup>, L.R. Osborne<sup>2</sup>, P.N. Ray<sup>1,2</sup>, J.M. Rommens<sup>1,2</sup>, A.D. Paterson<sup>1</sup>, D.E. Bulman<sup>3</sup>, S.W. Scherer<sup>1,2</sup>.* 1) The Centre for Applied Genomics and Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada; 3) Ottawa Health Research Institute, Ottawa, Ontario, Canada.

The Centre for Applied Genomics (TCAG; [www.tcag.bioinfo.sickkids.on.ca](http://www.tcag.bioinfo.sickkids.on.ca)) at The Hospital for Sick Children (Toronto) is a Canadian infrastructure established to facilitate innovative research and development in genetics and genomic biology. With the objective of providing core resources and technologies to support large-scale research projects, as well as for service-related research, five (unrestricted and not-for-profit) infrastructures operate. They are (i) Genome Resource Facility, (ii) Genetic Analysis Facility, (iii) Gene Expression Facility, (iv) DNA Sequencing and Synthesis Core, and (v) the Bioinformatics and Statistical Analysis Facility. Over the past year, 435 investigators from around the world used one or more of the facilities. To maximize application of these resources for the medical genetics community we have initiated a pilot project to integrate all cytogenetic breakpoint information with associated phenotypes into the human DNA sequence map. As a starting point we have collected and collated clinical records of over 1570 breakpoints and phenotypes from individuals with chromosome 7 rearrangements (850 constitutional and 720 malignancy-associated) and already localized over 500 of these to defined sites on the sequence map (see <http://www.chr7.org/>). Integrating this data with the DNA sequence and gene map has already led to the identification of candidate genes or mechanisms of disease for autism, split-hand split-foot syndrome, Williams-Beuren syndrome, cavernous malformation syndrome, splenic lymphoma, acute myeloid leukemia (see *Science* 300; 767-772, 2003); additional diseases will also be discussed. We are continuing to expand these studies by incorporating new chromosome 7 data sent from molecular and medical geneticists worldwide and are now considering a similar strategy to annotate the rest of the human genome.

**Subtelomeric FISH analysis of 1363 patients: A high frequency of subtle familial deletions and translocations.**  
S.A. Adams, A. Adeyinka, C.P. Lorentz, S.M. Jalal. Division of Laboratory Genetics, Mayo Clinic, Rochester, MN.

Subtelomeric DNA fluorescence *in situ* hybridization (FISH) probe panel is an effective tool for the investigation of children with developmental delay, idiopathic mental retardation, and nonspecific congenital anomalies. Of 1363 consecutive patients studied, one hundred eleven (111), or 8.14%, were abnormal. Of the abnormal results, 40 were derivative chromosomes, 53 were deletions, and 18 were balanced rearrangements or numeric abnormalities. The most common subtelomere abnormalities were those of 2p, 4p, 5p, 8p, and 10q. Cryptic subtelomere abnormalities of 12q, 15q, and 20q were observed and have not been previously reported. In addition, a high rate of familial abnormalities involving deletion and derivative chromosomes was identified. Among the patients with derivative chromosomes, 15 had family members tested. Of these, 10 (66.7%) inherited the abnormality from a parent who had a balanced translocation, while 5 (33.3%) were *de novo*. Among the deletion group, 21 had family members tested, and in 9 cases (42.8%) the deletion was inherited, whereas 12 (57.2%) were *de novo*. The most common familial abnormalities involved 3p, 4q, 7p, 8p, and 13q. In several families, the parent with the deletion had either normal clinical features or milder phenotype than the affected child. This chromosomal anticipation may be explained by several potential mechanisms, including unmasking of an autosomal recessive allele, imprinting effect, or expansion of the deletion in subsequent generations. To further elucidate this mechanism and to differentiate between normal variants and pathogenetic subtelomeric deletions, larger family studies need to be performed. The present findings underscore the need for parental FISH studies as part of the investigation of abnormal results, particularly because the rate of familial subtelomeric anomalies appears to be significantly higher than familial microdeletion syndromes and larger abnormalities detectable by banded chromosome analysis.

**Unbalanced Familial Subtelomeric Rearrangement Detected by FISH.** *S.A. Ebrahim<sup>1</sup>, A. Al Saadi<sup>2</sup>, K.S. Puder<sup>1</sup>, L. Gardner<sup>1</sup>, A. Johnson<sup>1</sup>.* 1) Dept Pathology, Cytogenetics, Wayne State Univ, Detroit Medical Center Detroit, MI; 2) Dept Anatomical Pathology, Beaumont Hospital, Royal Oak MI.

A subtle familial unbalanced rearrangement involving the short arm of chromosome 18 was identified in a woman, her son and unborn fetus. Standard chromosome analysis revealed a subtle 18p11.3 deletion that was later diagnosed by subtelomeric FISH probes as a cryptic unbalanced 5;18 translocation. The proband, a 3 year old Caucasian male, was referred for chromosome analysis due to multiple anomalies. Lymphocyte chromosome analysis revealed a male karyotype with a subtle distal 18p deletion. FISH analysis using a probe for the 18p subtelomeric region confirmed the loss of 18p11.3-pter region. Testing of the mother during her next pregnancy confirmed that she was a carrier of the same 18p deletion. High-resolution ultrasound at 19 weeks of gestation showed no apparent fetal anomalies, however, cytogenetic and FISH analysis of amniocytes revealed the same subtle distal 18p deletion. The patient was counseled regarding these findings, and opted to continue the pregnancy. The maternal grandparents were unavailable for testing. To further evaluate the familial deletion, analysis with a subtelomeric FISH probes was performed. The proband and his mother were found to have a previously undetected gain of 5p subtelomeric material translocated distal to 18p. The mother and her sibs are therefore monosomic for 18p11.32 and trisomic for 5p15.33. Deletion of 18p has been reported, with a wide range of phenotypic variability. The majority of cases are sporadic, however, about 15% are familial. It is likely that a subset of cases reported as familial deletion are cryptic rearrangements involving other subtelomeric regions. For proper genetic counseling, a careful evaluation of subtelomeric FISH probes assay should be included in the diagnostic work-up of patients with apparent familial chromosome deletion syndromes.

**Subtelomeric rearrangements and mental retardation: further phenotypic definition.** *K. Anyane-Yeboah<sup>1</sup>, T. Jancelewicz<sup>1</sup>, W. Chung<sup>1</sup>, E. Cox<sup>1</sup>, E. Guzman<sup>1</sup>, L. Tambini<sup>2</sup>, D. Warburton<sup>1</sup>.* 1) Division of Genetics -BHN6-601A, New York Presbyterian Hospital(Columbia University), New York, NY; 2) Dept of Obstetrics and Gynecology, St Luke's-Roosevelt Hospital, New York, NY.

Cryptic subtelomeric rearrangements have been reported in 4-7% of moderate to severely retarded individuals with apparent normal chromosomes(Knight et al,1999;deVries et al,2001). We present phenotypic information on 12 children from 11 families with cryptic subtelomeric chromosome rearrangements detected by FISH in our clinic population of moderate to severely retarded children with apparently normal chromosomes. The rearrangements were made up of the following deletions: 1p(2 cases), 3q, 9p, 9q(1), 22q(2 cases) and unbalanced translocations: der(4)t(4;5)(p;p), (8)t(8;10)(p;p), (9)t(9;1)(q;p), (9)t(9;16)(q;p), (22)t(22;16)(q;q). 3 balanced translocation carrier parents were identified in 8 families studied(37.5%). 41% of deliveries were via C-section and 33% of the pregnancies were associated with complications. Ten pregnancies were term, 1 preterm and 1 postdated. Apgar scores were normal in all cases. 33% were IUGR and 42% were born microcephalic. 50% overall subsequently became microcephalic. Feeding difficulty in infancy was present in 72%. Cognitive deficits were present in all, but was severe to profound in 83%. 33% had no speech and in an additional 50% speech was severely delayed. Muscle tone was abnormal in 75% and behavior was aggressive or hyperactive in 42%. There was history of seizures in 33%, congenital heart disease in 66%, hypertelorism in 66% and hypothyroidism in 16%. Abnormalities of skin including wrinkled palms and soles, hemangiomas, nevi and cutis marmorata were present in 42%. Abnormalities of the hand and feet, generally minor were present in 66% and included persistent fetal pads, small hands and feet, clinodactyly and minor overlap of the toes. This analysis is an attempt to define a phenotype that will be helpful to clinicians in the selection of suitable patients for subtelomeric FISH analysis in order to maximize yield of positive results and minimize costs.

**High resolution FISH analysis of NF1 REP and non-REP microdeletions evidences nonhomologous end joining mediated rearrangements.** *P. Riva<sup>1</sup>, M. Venturin<sup>1</sup>, C. Gervasini<sup>1</sup>, F. Orzan<sup>1</sup>, A. Bentivegna<sup>1</sup>, L. Corrado<sup>1</sup>, P.*

*Colapietro<sup>1</sup>, A. Friso<sup>2</sup>, R. Tenconi<sup>2</sup>, M. Upadhyaya<sup>3</sup>, L. Larizza<sup>1</sup>.* 1) Dept of Biology & Genetics, Medical Faculty University of Milan, Italy; 2) Clinical Genetics and Epidemiology Unit, Dept of Pediatrics, University of Padua, Italy; 3) Inst of Medical Genetics, University of Wales College of Medicine, Cardiff UK.

NF1 microdeletion syndrome is caused by haploinsufficiency of the NF1 gene and its flanking regions. NF1 patients carrying gross deletion, often displaying complex phenotype, account for 5-10% of all NF1 mutations. Most of the NF1 deletions (80%) originate by unequal homologous recombination of repeated sequences (REP-P and M) mapped to 17q11.2, while the remaining show unusual breakpoints. We performed high-resolution FISH analysis of 20 NF1 microdeleted patients with the aim of mapping non recurrent deletion breakpoints and verifying the presence of other recombination-prone genome architectural motifs. Conventional FISH analysis allowed us to identify 17 REP-deleted patients, and three patients carrying atypical deletions of 1.3 Mb, 1.5 Mb and 3 Mb respectively. By fiber-FISH, we identified genomic intervals of ~100 Kb comprising the breakpoint regions, which proved to be affected by reported deletions and translocation. The generation of several locus-specific FISH probes allowed us to restrict the critical deletion endpoint intervals to a few Kb. This approach led us to hypothesize that small blocks of REPs cluster around the 1.3 Mb deletion breakpoints and LPA1 repeats may be involved in the 1.5 Mb deletion breakpoints. The precise restriction of the deletion interval, by locus-specific probe, allowed us to isolate the 3 Mb deletion junction fragment by long-range PCR. Sequencing of the novel junction fragment indicated that the deletion is likely caused by non homologous end joining. We also established the exact deletion gene content in non-REP deletions which, by comparison with the REP deletion gene content, will contribute to carrying out genotype-phenotype correlation studies.

**De novo 14q subtelomere deletion and 15q subtelomere duplication in a child: detection by FISH subtelomere analysis.** *N. Qin<sup>1</sup>, M.G. Bialer<sup>2</sup>, I. Taff<sup>3</sup>, S. Hahn<sup>2</sup>, D. LaGrave<sup>1</sup>, B.J. White<sup>1</sup>.* 1) Cytogenetics, Quest Diagnostics Nichols Ins, San Juan Capistrano, CA; 2) Div of Med Genetics and; 3) Ped Neurology, North Shore Univ Hosp/NYU Med Center, Manhasset, NY.

An 8 yr old white girl presented with hypotonia, developmental delay, and dysmorphic features. She had been delivered by C-section at 37 wk gestation because of fetal bradycardia and failure to progress. Her birth weight was 3040 g. She was polycythemic at birth and severely constipated as a neonate. Mild hypotonia, epicanthal folds, a narrow palate, and retrognathia were noted at 6 mo. At 12-15 mo, speech and motor development were delayed. A DNA methylation test for Prader-Willi syndrome was normal, as were metabolic studies and a DNA test for myotonic dystrophy. She has been diagnosed with ADD and probably has mild mental retardation. She has fractured or lost 4 teeth and her extremities sometimes turn red or blue. At 8 yr, her height was 129.1 cm (25-50th percentile), weight 22.5 kg (10th percentile), and OFC 50 cm (~10th percentile). She had dysmorphic features including a long, thin face, medial flaring of the eyebrows, a high, narrow nasal bridge, malar hypoplasia, a narrow, high arched palate, a long, recessed jaw, dental crowding, a long philtrum and prominent triangular anhelices. Mild joint limitation, tapered fingers, and soft neurological signs were evident. High-resolution chromosome analysis and a FISH microdeletion syndromes panel were normal. However, on FISH subtelomere analysis, one 14q subtelomere signal was absent, replaced by an additional 15q subtelomere signal; the resulting imbalance consists of a 14q subtelomeric deletion and a 15q subtelomeric duplication. Parental 14q and 15q FISH subtelomeric probe findings were normal, suggesting a de novo rearrangement. To our knowledge, coexistence of these chromosome abnormalities has not been reported before. However, this patient's presentation is more consistent with 14q deletion than with 15q duplication. Our results add to knowledge on clinical findings in subtelomeric deletions and duplications and demonstrate the value of FISH subtelomere screening in children with unexplained dysmorphisms and developmental delay.

**Subtelomere FISH: Identification of Familial Variants.** *J.H. Tepperberg, I.K. Gadi, P.N. Mowrey, S.K. Griffin, C.J. Bullen, P.J. Schupp, P.D. Singh-Kahlon, L. Wisniewski, P.R. Papenhausen.* Dept Cytogenetics, Laboratory Corp of America, Res Triangle Pk, NC.

Unbalanced subtelomere alterations have generally been associated with developmental delay, minor dysmorphic features and mild mental retardation. In over 2500 cases, we have observed 10 familial unbalanced autosomal subtelomere alterations. The proband in each case was referred because of phenotypic abnormalities, developmental delay and/or mental retardation. Three cases showed a 2qter deletion detected with the CytoCell multiprobe subtelomere panel. In each case, a parent was found to have the same deletion using the same DNA probe. Analysis with a slightly more proximal subtelomere DNA probe (Vysis, Inc.) showed this region to be present in all individuals. Three unrelated cases showed partial trisomy 4pter from a der(22)t(4;22)(pter;pter) translocation. Follow-up parental studies showed the same unbalanced alteration. Two cases showed diminished subtelomere DNA hybridization signals. The first case showed a diminished hybridization signal for distal 4p and the second case showed a reduced signal for 10q. Subsequent paternal analyses showed a diminished hybridization signal, for the respective chromosome, in both cases. An alteration of chromosome 17pter with an unexplained green (FITC) hybridization fluorochrome color was observed in both the proband and mother. A rearrangement involving chromosome 8p, which has a FITC fluorochrome in the Vysis mix with 17p, was ruled out with a specific chromosome 8p subtelomere probe. Finally, a 21q subtelomere deletion was observed in a young girl and her phenotypically normal mother. The presence of the same alteration in the proband and parent would suggest that the specific DNA probes are targeting non-coding DNA sequences. Alternatively, it is conceivable that an abnormal recessive gene is unmasked by a knockout of the normal allele in the proband consequently leading to an abnormal phenotype. Therefore, conclusions based strictly on subtelomere studies should only be interpreted within the context of a parental subtelomere analysis.

**Detection of trisomy 21 and other aneuploidies by Paralogous gene quantification.** *S. Deutsch*<sup>1</sup>, *U. Choudhury*<sup>1</sup>, *A. Sylvan*<sup>2</sup>, *S.E. Antonarakis*<sup>1</sup>. 1) Division of Medical Genetics, University of Geneva, Geneva, Switzerland; 2) Pyrosequencing AB, Uppsala, Sweden.

The link between Down Syndrome and trisomy of human chromosome 21 was established in 1959, and since then diagnosis of aneuploidies has been performed mostly by Karyotyping and later FISH, two methods that are based on counting the number of chromosomes present in human cells. These approaches are highly accurate but costly and labour intensive. The development of alternative methods for faster, higher throughput, accurate and semi-automated diagnosis of common cytogenetic abnormalities would be highly desirable. We have developed a simple method for the detection of chromosome number abnormalities based on the use of paralogous genes. These are genes that have a common evolutionary origin but have been duplicated over time in the human genome. They are in many cases located on different chromosomes, and show a high degree of sequence similarity. We exploit this sequence similarity in order to co-amplify paralogous sequences located on different chromosomes with identical primer pairs. Single nucleotide differences between the paralogue sequences which we call "Paralogous sequence differences" or PSDs, are then quantified using the Pyrosequencing technology, and the ratio of the PSDs reflects the relative copy-number of the chromosomes tested. We have now developed and validated tests for trisomies of chromosomes 21, 13 and 18, as well as all X and Y chromosome abnormalities in over 100 patients. At least two independent tests have been developed for each chromosomal abnormality. In addition we have used the same principle to detect common deletions such as those in DiGeorge and Williams-Beuren syndromes. For all tests, the correct diagnosis for each DNA was unambiguously assigned. This method has advantages over the microsatellite-based detection of aneuploidies, since the tests work on all DNAs (irrespective of informativeness of polymorphisms), and the detection of deletions does not require the parental DNAs.

**PHENOTYPE AND BEHAVIOR IN PATIENTS WITH ANGELMAN SYNDROME: COMPARISONS BETWEEN THE DIFFERENT CLASSES OF DELETIONS AND BETWEEN PATIENTS WITH A**

**DELETION AND WITH UPD.** *M.C. Varela<sup>1</sup>, F. Kok<sup>2</sup>, I. Kohl<sup>1</sup>, C.I.E. Castro<sup>1</sup>, C.P. Koiffmann<sup>1</sup>.* 1) Dept of Biology, Bioscience Institute, University of Sao Paulo; 2) Child Neurology Service, Dept of Neurology, Hospital das Clinicas, University of Sao Paulo, Sao Paulo, Brazil. email: [mcvarela@ib.usp.br](mailto:mcvarela@ib.usp.br). Support: FAPESP, CNPq.

The Angelman syndrome (AS - developmental delay, severe mental retardation, speech impairment, ataxia, outbursts of laughter, seizures) can result from either a 15q11-q13 deletion (del), paternal uniparental disomy (UPD), imprinting, or UBE3A mutations. We describe here the phenotypic and behavioral variability in 48 patients with different classes of deletions (11 BP1-BP3, 14 BP2-BP3, 2 BP2-BP4, 1 BP2-BP5, 20 inconclusive) and 9 patients with UPD. The diagnosis was made by methylation pattern analysis of the SNRPN-SNURF gene (exon1) and microsatellite analysis of loci within and outside the PWS/AS region. The clinical pictures of the patients with different classes of deletions showed no major phenotypic differences. Frequent swallowing disorders, brachycephaly, some capacity of communication and hyperactivity were more prevalent in BP2-BP3, while absence of speech was more common in BP1-BP3 patients. Previous reports have indicated that in patients with UPD the AS phenotype was milder than in patients with deletions, children with UPD had a better physical growth, fewer or no seizures, less ataxia and higher cognitive skills. Here in deletion patients presented a higher incidence of swallowing disorders (in centile) (73.9del;40UPD), hypotonia (71del;16.6UPD), absence of speech (91.3del;66.6UPD), microcephaly (57.7del;11.1UPD), and outbursts of laughter (95.74del;77.7UPD). Besides the abnormalities already described, weight (55.5UPD;17.9del), height (44.5UPD;11.4del), and OFC (33.3UPD;2.22del) above the 75th centile were more frequent among the UPD patients. These findings confirm the hypothesis that the severe phenotype of deleted patients is caused by haploinsufficiency of other genes in the deleted region. The frequency of UPD in AS patients reported in the literature is 2-3% of all AS cases, and, since these children may present a less typical phenotype, we suggest that AS patients with UPD may remain undiagnosed.

**Commercial FISH probes for Smith-Magenis syndrome do not contain the *RAII* gene and fail to diagnose all cases.** *C.N. Vlangos*<sup>1</sup>, *J. Blancato*<sup>4</sup>, *M. Wilson*<sup>5</sup>, *A.C.M. Smith*<sup>6</sup>, *S.H. Elsea*<sup>1,2,3</sup>. 1) Genetics Graduate Program; 2) Dept of Zoology; 3) Dept of Pediatrics and Human Development, Michigan State Univ, East Lansing, MI; 4) Institute for Molecular and Human Genetics, Georgetown Univ, Washington, DC; 5) Dept of Clinical Genetics, The Children's Hospital at Westmead, Westmead, Australia; 6) National Human Genome Research Institute, NIH, Bethesda, MD.

Smith-Magenis syndrome (SMS) is a multiple congenital anomalies/mental retardation disorder usually 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 SMS deletion occurs in ~70% of deletion patients and spans ~4 Mb, while the critical SMS region spans ~900 kb and contains ~25 genes. SMS was thought to be a contiguous gene syndrome, though recently we determined that the core SMS features are due to haploinsufficiency of the *RAII* gene, which maps to the middle of the SMS critical region. All SMS patients carrying a deletion are deleted for *RAII*. Currently, diagnostic testing for the SMS deletion relies on commercial probes mapping to the proximal side of the SMS critical region and do not include *RAII*. We present here two patients with the typical SMS phenotype who carry a cytogenetically visible 17p11.2 deletion. An SMS deletion was not detected using commercially available FISH probes in these patients. Further analysis using FISH probes mapping to the central portion of the critical region show that these patients are deleted for *RAII*. These findings further narrow the SMS critical region and provide additional evidence that haploinsufficiency of the *RAII* gene is the cause of the core SMS phenotype. Current commercial FISH probes cover the proximal end of the SMS critical region and may provide false negative results for diagnosis of SMS. Diagnostic testing for SMS should utilize FISH probes containing the *RAII* gene in all new cases. Furthermore, patients testing negative with currently available FISH probes should be re-evaluated using an *RAII* containing probe. Patients negative for *RAII* FISH may be appropriate for mutation screening.

**Cryptic chromosomal rearrangement screening in 30 patients with mental retardation and dysmorphic features.**

*M. Mila<sup>1,2</sup>, L. Rodriguez-Revenga<sup>1,2</sup>, C. Badenas<sup>1,2</sup>, A. Sanchez<sup>1,2</sup>, J. Mallolas<sup>1</sup>, A. Carrio<sup>1,2</sup>, National Network on Mental Retardation.* 1) Serv Genetica, Hospital Clinic, Barcelona, Barcelona, Spain; 2) IDIBAPS (Institut d'Investigacions Biomediques Agusti Pi i Sunyer). Hospital Clinic. Barcelona. Spain.

Mental retardation affects 1-3% of the general population and the genetic causes in many cases are unknown. Cytogenetically invisible chromosomal imbalances have been indicated as an explanation for them. Nowadays, and due to the development of molecular cytogenetic techniques, it is possible to identify cryptic rearrangements involving the ends of chromosomes. Over 500 children with moderate to severe mental retardation have been screened for their telomeres and it has been established that 5-10% of these have subtle abnormalities of chromosome ends. The clinical consequences of different telomere alterations are mostly unknown and with this type of study new syndromes are being delineated. We report a screening using chromosome-specific telomere FISH probes in a group of 30 patients with a well-characterized phenotype including mental retardation, dysmorphic features and normal karyotype. Among them, two subtelomeric rearrangements have been detected and characterized, the frequency of imbalances is estimated to be 6.7%. One of them is a de novo deletion of 1p36, which has been previously described as a contiguous gene syndrome. The second one is an unbalanced product of a cryptic translocation involving chromosomes 1 and 13, which results in a partial 1q trisomy and partial 13q monosomy. It is interesting to define a genotype-phenotype correlation for telomere regions in order to identify dosage-sensitive genes involved in human genetic diseases. It is worth highlighting the importance of searching for cryptic subtelomeric rearrangements in nonsyndromic mentally retarded patients in order to determine the etiology of mental retardation. Acknowledgements: Red de Grupos V2003-redG098-O.

**Fast routine screening for subtelomeric deletions and duplications using Multiplex Ligation-dependent Probe Amplification (MLPA).** *E. Sijtermans, C. de Kovel, D. Smeets, C. van Ravenswaay, B. de Vries, H. Brunner, W. Nillesen.* Dept Human Genetics, Univ Medical Centre Nijmegen, PO-Box 9101, 6500 HB Nijmegen, The Netherlands.

Subtelomeric submicroscopic rearrangements are known to be the cause of mental retardation in ~5% of patients. As a result, there is a demanding need for a fast, inexpensive and reliable detection method in routine diagnostic labs. Several methods, such as multiprobe FISH analysis, Multiplex Amplifiable Probe Hybridization (MAPH), and array-CGH do not meet all the criteria stated above. Recently a new technique was developed to analyse copy-numbers of almost any given chromosomal segment. This technique, Multiplex Ligation-dependent Probe Amplification (MLPA) is based on the annealing and subsequent ligation of two adjacent primers, followed by a quantitative PCR step (for technical details see [www.mrc-holland.com](http://www.mrc-holland.com)). MLPA is now routinely used for the detection of exon deletions in several human genes, including *BRCA1*, *MSH2* and *MLH1*. Recently a first generation MLPA probe-set for the detection of subtelomeric deletions/duplications became available. We have validated this set and subsequently analyzed 180 patients suspected of chromosomal aberrations but with a normal karyotype. Although the kit is not yet optimal for all chromosome ends (at least 3 probes need to be replaced, until now ~60% could be validated on patients with known deletions), anomalies have been detected in almost 8% of the patients. We are now in the process of confirming these findings by FISH analysis or array-CGH. These results indicate that MLPA (upon further optimization of the probe-sets) is likely to become the method-of-choice for routine analysis to screen for subtelomeric deletions.

**Detection of cryptic subtelomeric rearrangements in idiopathic syndromic mental retardation using telomeric QMPSF, a new molecular genetics method.** *P. Saugier-veber<sup>1,2</sup>, C. La Rochebrochard<sup>1,2</sup>, N. Le Meur<sup>2</sup>, A. Rossi<sup>3</sup>, V. Layet<sup>4</sup>, V. Drouin-Garraud<sup>2</sup>, A. Goldenberg<sup>2</sup>, G. Joly-Hélas<sup>2,5</sup>, H. Moïrot<sup>2,5</sup>, M. Tosi<sup>1</sup>, T. Frebourg<sup>1,2</sup>.* 1) INSERM EMI-U 9906, Faculty of Medicine 76183 Rouen, France; 2) Department of Genetics, CHU de Rouen, 76031 Rouen, France; 3) Department of Genetics, Etablissement Français du Sang, 76235 Bois-Guillaume, France; 4) Cytogenetics, Centre Hospitalier du Havre, 76083 Rouen, France; 5) Cytogenetics, CHU de Rouen, 76031 Rouen, France.

It is estimated that 6 % of all cases of idiopathic syndromic mental retardation may result from cryptic subtelomeric rearrangements. Most laboratories use FISH with telomere specific probes, but this method remains difficult, expensive and time consuming for routine diagnostic tests. CGH, microarray-CGH or microsatellites studies have been described but remain incompatible with high-throughput diagnosis. We developed a new simple molecular genetics assay based on the QMPSF (Quantitative Multiplex PCR of Short Fluorescent Fragments) method, based on the simultaneous amplification of short fluorescent genomic fragments. For each subtelomeric end, we choose an exonic amplicon of 160-300 bp within the BAC or PAC used for the FISH assay and, when possible, a second more telomeric amplicon. Thus, for the 41 telomere exploration, 46 amplicons were used and integrated into 9 multiplex PCR. This method was first validated with 25 samples with characterized subtelomeric rearrangements. We have initiated the screening of 500 patients presenting with idiopathic syndromic mental retardation, and of 150 normal controls in order to document the existence of polymorphic rearrangements. We already detected a 15qtel duplication and a 19qtel deletion in two patients as well as a 10qtel duplication in a control. Telomeric QMPSF may constitute a simple method for the detection of cryptic subtelomeric rearrangements in patients with idiopathic mental retardation. Furthermore, because of its flexibility, telomeric QMPSF may facilitate the cartography of the rearrangements, the molecular cloning of the breakpoints and the positional cloning of genes involved in mental retardation, or malformations.

**Molecular mechanisms in Xp22-p21 deletions among patients with complex glycerol kinase deficiency (GKD).** *Y.-H. Zhang<sup>1</sup>, J.C. Ho<sup>2</sup>, B.-L. Huang<sup>1</sup>, L.L. McCabe<sup>1,2</sup>, K.M. Dipple<sup>1,2,3</sup>, E.R.B. McCabe<sup>1,2,3</sup>.* 1) Department of Pediatrics, David Geffen School of Medicine at UCLA, LA, CA; 2) Department of Human Genetics, David Geffen School of Medicine at UCLA, LA, CA; 3) Mattel Children's Hospital at UCLA, LA, CA.

Chromosomal rearrangements in Xp22-p21 typically involve the adrenal hypoplasia congenital (AHC), glycerol kinase (GK) and/or Duchenne muscular dystrophy (DMD) loci. The deletion sizes and breakpoints are unique within each family. The purpose of our investigation was to sequence patients deletion breakpoints and to identify potential mechanisms responsible for these recombination events. The breakpoints were sequenced using an ABI 3700 automated sequencer, and the sequences were analyzed using the NCBI and UCSC websites. We sequenced the breakpoints in six affected males (A-F) and identified the following: A) 0.4Mb interstitial deletion involving AHC; B) 0.6Mb interstitial deletion involving AHC and GK; C) 0.7Mb interstitial deletion involving GK and DMD; D) 1.9 MB interstitial deletion involving AHC and GK; E) 3.2 Mb interstitial deletion involving GK and DMD; and F) 4.5 MB interstitial deletion involving AHC, GK and DMD. The patients phenotypes matched the observed deletions for all six patients. Among the six patients, the data showed one Alu-Alu recombination, and three nonhomologous end-joining (NHEJ) events with 2-3 bp micro-homologies. The remaining two involved Alu sequence insertions. Our data are consistent with previous results from rearrangements involving other chromosomes. Unequal crossing over may occur between repeat sequences and very short NHEJ overlaps of 1-5 bp have been described in recombinant chromosomes. The possibility of retrotransposition events remains for both of the deletions associated with Alu insertions. In summary, the recombination breakpoints in these six patients with Xp22-p21 rearrangements differ from each other and potential mechanisms are consistent with observations of similar events elsewhere in the human genome.

**Refined risk of UPD associated with prenatally-detected inv dup(15) chromosomes.** *B. Huang*<sup>1,2</sup>, *A.N. Lamb*<sup>3</sup>. 1) Genzyme Genetics, Orange, CA; 2) Division of Human Genetics, University of California Irvine; 3) Genzyme Genetics, Westborough, MA.

Marker chromosomes originating from chromosome 15, often referred to as inv dup(15), are the most common marker chromosomes found in humans. The large marker 15 chromosomes that contain the Prader-Willi syndrome (PWS)/Angelman syndrome (AS) critical region are usually associated with an abnormal phenotype and the small inv dup(15) chromosomes that do not contain the PWS/AS region are mostly associated with a normal phenotype. The observation of the PWS or AS phenotype in a small minority of these patients has been mainly due to the presence of uniparental disomy (UPD) for chromosome 15. The risk of UPD in prenatally-detected de novo inv dup(15) cases has been reported to be as high as 12% (2 in 17 cases) in a previous study. In this report, we attempt to further define the risk associated with the prenatally-detected inv dup(15)s.

A total of 37 cases of inv dup(15)s identified during prenatal diagnosis that did not contain the PWS/AS region were examined for the presence of UPD for chromosome 15. Among the 37 cases, the inv dup(15) chromosomes were de novo in 23 cases, familial in 11 cases, and inheritance was unknown in 3 cases. In 35 cases, UPD was ruled out prenatally by either methylation analysis or microsatellite analysis. UPD was excluded in the remaining 2 cases by the presence of a normal phenotype during postnatal follow-up. Therefore, UPD was not detected in these 37 cases. Combining the previous study with this study, the overall risk of UPD associated with the de novo inv dup(15) chromosomes identified during prenatal diagnosis is lowered to 5% (2 in a total of 40 de novo cases). Further study with a larger number of cases is necessary for more accurate risk assessment.

**Single-Tube Polymerase Chain Reaction Analysis of 14-Microsatellite Markers for the Detection of Uniparental Disomy of Chromosome 15.** *S. Wang, F. Quan, W. Sun, C.M. Strom.* Dept Molecular Genetics, Quest Diagnostics Nichols Inst, San Juan Capist, CA.

Both Prader-Willi (PWS) and Angelman (AS) Syndromes are complex genetic disorders with severe clinical manifestations, including developmental delay, mental retardation, and dysmorphic features. About 70% of PWS cases are caused by a microdeletion of paternal chromosome 15q11.2-q13. Approximately 25% of PWS cases are caused by maternal uniparental disomy for chromosome 15 (UPD 15). Approximately 70% of AS cases are caused by a microdeletion of maternal chromosome 15q11.2-q13, and 3-5% of AS cases are caused by paternal UPD 15. Here we report a single-tube multiplex PCR-based microsatellite assay to detect UPD 15. The single-tube PCR assay analyzes 14 microsatellites, including 6 FAM-labeled (D15S988, D15S205, D15S986, D15S118, D15S130 AND D16S515), 3 VIC-labeled (D16S423, D15S978 and D16S3103), and 5 NED-labeled (D15S1007, D15S120, D15S128, D16S516 and D15S994) microsatellites. The heterozygosity of these microsatellites ranges from 0.66 of 0.88. To analyze microsatellites, we employed a highly automated system for sizing fluorescent-tagged PCR products. This system consisted of 1) capillary electrophoresis of PCR products on an Applied Biosystems (ABI) 3100 Genetic Analyzer, 2) fragment sizing using an internal size standard (GS 500Liz) and Genescan Analysis Software, and 3) genotype determination using the Genotyper Software. This 14-plex PCR assay can successfully genotype DNA samples with 2 to 100 ng genomic DNA in a 15 l reaction. Due to the discriminatory power of the large number of highly polymorphic microsatellites analyzed, we believe this multiplex PCR assay can determine the parental origin of chromosome 15 for all patient samples and thus enable the detection of UPD 15 for the diagnosis of PWS and AS.

**Random X-inactivation in an SRY positive, XX male.** *A. Al Saadi.* Dept Anatomic Pathology, William Beaumont Hosp, Royal Oak, MI.

Inactivation of an X chromosome in females is usually random, with some exceptions. Genes from pseudoautosomal and other regions on the human X chromosome usually escape inactivation. We report here a case of a 13-year-old, who was diagnosed prenatally as a female, 46,XX, but was phenotypically male at birth. The patient has been monitored regularly from early childhood. On his last visit, he appeared normal for his age and showed no evidence of hormonal deficiencies. His pubertal development has been normal without assistance. DHEA - sulfate was 64, LH 1.0, FSH 0.8, testosterone 46, his bone age and all other clinical evaluations were normal. He was referred to our laboratory for chromosomal analysis, since none was done post-natally. Cytogenetic studies were performed on both blood leukocytes and skin fibroblasts. A mosaic karyotype 45,X/46,XX constitution was found in both tissues. Eighteen of 50 leukocytes and 12 of 50 skin fibroblasts were 45,X. FISH study, using SRY DNA probe revealed that one of the X chromosomes in the 46,XX line and the single X in the 45,X line had positive signals.

X-inactivation study revealed a random inactivation in the 46,XX cells, which were previously scored for the SRY signal in both tissues. Since there was no mention of mosaic karyotype in the prenatal diagnostic study, it is proposed that the normal X chromosome is selectively lost, and that with the SRY is conserved in the 45,X line. The random inactivation in the 46,XX line is unlikely to involve the pseudoautosomal region with SRY. Normal hormonal and physical development in the 46,XX males may require that the SRY region be active in all cells. Molecular studies are underway to evaluate the SRY expression in the active and inactivated X chromosomes of the 46,XX line in this patient.

**Functional disomy of Xp: prenatal findings and postnatal outcome.** *K. Godbole<sup>1</sup>, E. Kolomietz<sup>2</sup>, E.J.T. Winsor<sup>2</sup>, D. Chitayat<sup>1,3</sup>*. 1) Dept Clinical Genetics, Hosp Sick Children, Toronto, ON, Canada; 2) Department of Laboratory Medicine and Pathobiology, Mount Sinai Hospital, Toronto, ON, Canada; 3) Prenatal Diagnosis and Medical Genetics, Mount Sinai Hospital, Toronto, ON, Canada.

We report a trisomy of the short arm of X chromosome (Xp11.2-pter) due to a de novo unbalanced X;13 translocation diagnosed prenatally in a female fetus. Amniocentesis was performed at 20 weeks gestation following the finding of Dandy-Walker malformation on a prenatal ultrasound examination. The trisomy of Xp11.2-pter was confirmed with fluorescence in situ hybridization (FISH), using an X chromosome painting probe and telomeric FISH probes specific for the short arm of chromosome X. The karyotype was defined as 46,XX,der(13)t(X;13)(p11.2;p11.2). FISH analysis with an XIST probe showed that the derivative chromosome 13 does not include the XIST locus at the X-inactivation center. A guarded prognosis for growth and development was discussed with the couple and they chose to continue the pregnancy. A severe phenotype was seen at birth including macrosomia, facial dysmorphism with preauricular tag, congenital heart disease and structural brain malformations. Because the derivative chromosome was not subject to X inactivation, functional disomy of Xp11.2-pter most likely accounts for the abnormal phenotype in this patient.

**Skewed X-chromosome inactivation in Premature Ovarian Failure.** *K.L. Bretherick<sup>1</sup>, M.R. Fluker<sup>2</sup>, C.L. Beever<sup>1</sup>, C.L. Anderson<sup>1</sup>, C.J. Brown<sup>1</sup>, W.P. Robinson<sup>1</sup>.* 1) Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Genesis Fertility Centre, Vancouver, BC, Canada.

Although the average age of menopause in humans is 51 years, approximately 1% of women will experience menopause before the age of 40, a condition known as Premature Ovarian Failure (POF). Known genetic causes of POF include various X-chromosome mutations and deletions, however in most cases the genetic etiology remains unknown. In females, an X-chromosome carrying a disadvantageous mutation or deletion is often preferentially inactivated resulting in skewed X-chromosome inactivation (XCI). We therefore examined XCI skewing in women with idiopathic POF to determine if undetected X-chromosome mutations or microdeletions are a significant cause of POF. XCI skewing was assayed by methylation-sensitive restriction enzyme digestion and PCR amplification of the polymorphic X-chromosome markers, AR and FMR1. XCI skewing using the AR assay was similar in cases and controls; 3 of 36 (8.3%) women with POF and 7 of 94 (7.4%) control women had extremely (90%) skewed XCI ( $p=0.6$ ), suggesting that X-chromosome mutations and deletions causing skewed XCI do not account for the cases of POF in our population. However, women with POF were more skewed using the FMR1 assay than they were with the AR assay; of 7 POF cases with a difference in skewing of 10% between the FMR1 and AR assays, 6 (85%) were more skewed using FMR1 than AR. This was not seen in controls; of 33 controls that had a 10% difference in skewing between the assays, 14 (42%) were more skewed using the FMR1 assay ( $p=0.09$ ). The increase in skewing detected using the FMR1 assay may reflect the role of FMR1 in POF; FMR1 premutation size alleles have been associated with POF. Interestingly, the distribution of skewing in women with POF shows a bias towards demethylation of the larger FMR1 allele ( $N=27$ , skewness of distribution = -0.27), a trend that is not so apparent in controls ( $N = 70$ , skewness of distribution = -0.12). This may represent a tendency for women with POF to activate larger FMR1 alleles, a finding of potential importance in elucidating the etiology of the FMR1 association with POF.

**Chick neural tube electroporation for the analysis of Zic2 function in CNS development.** *L. Brown, S. Brown.*  
Ob/Gyn, Columbia Univeristy , New York, NY.

Loss-of-function mutations in *ZIC2* result in severe brain malformations in humans and mice, proving that the developing CNS is very sensitive to the level of *Zic2* gene product. Our own unpublished data show that ectopic expression of *Zic2* in the developing spinal cord of mice results in severe malformation, confirming this idea. In order to pursue this observation and to further investigate how *Zic2* acts at the molecular level, we have used chick neural tube electroporation as a rapid and inexpensive method for studying the mis-expression of genes in the developing spinal cord.

In this system, 48 hour chick embryos are exposed by un-roofing the egg and plasmid DNA is injected into the open neural tube using a fine glass micropipette. A train of pulses of DC current is then applied through platinum electrodes placed on either side of the neural tube. The egg is then allowed to incubate for up to 72 hours before it is sacrificed and prepared for either whole-mount analysis of gene expression or for serial sectioning. The use of green fluorescent protein (GFP) allows for a rapid assessment of electroporation efficiency. Because the DNA only enters one half of the neural tube, the contra-lateral side serves as a convenient control for all downstream gene expression studies.

We have used this system to express both normal and mutant mammalian alleles of *Zic2*. Interestingly, at 48 hours after electroporation, there is no discernable expression of *Zic2* although an inactive *Zic2* allele is expressed without problem as is GFP. By examining earlier time points, we were able to demonstrate that electroporation of *Zic2* results in rapid apoptosis as determined by TUNEL assay and staining for anti-phospho-histone H2AX. The use of mutant alleles of *Zic2* indicates that apoptosis depends on the presence of an intact DNA binding domain but not transactivation activity of the *Zic2* protein. These results point the possibility that *Zic2* has a very divergent roles in chick and mammalian development. Work is currently in progress to clone the full length *Zic2* protein.

**Expression profiles of regenerating avian hair cells.** *R.D. Hawkins<sup>1</sup>, S. Bashirdes<sup>1</sup>, M.E. Warchol<sup>2</sup>, M. Lovett<sup>1</sup>.* 1) Genetics, Washington Univ, St Louis, MO; 2) Central Institute for the Deaf, St Louis, MO.

Age-related hearing loss affects one-third of people over the age of 50, and fifty percent of those over the age of 60. While the environment and/or genetic predisposition are causative, a major physiological limitation is the inability to regenerate damaged hair cells in the inner ear. These specialized cells are necessary for hearing and balance. The avian inner ear, unlike that of mammals has the remarkable ability to regenerate damaged hair cells. After exposure to ototoxic drugs or oto-acoustic trauma, damaged hair cells re-grow by proliferation and differentiation of supporting epithelial cells. In the current study cultured utricular and cochlear sensory epithelial cells, hair cells and supporting cells, were treated with an ototoxic drug, neomycin, for 24 hours (hrs), and allowed to recover for 0hrs, 24hrs, and 48hrs. Each time point was profiled for changes in gene expression compared to time-matched controls. Earlier time points were also profiled from laser damaged utricular sensory epithelia at 30mins, 1, 2, and 3hrs. Due to the small number of cells, a micro-cDNA amplification method was employed to generate enough labeled target for expression profiling. Time point samples were hybridized to custom oligonucleotide arrays that interrogate ~1700 human transcription factor genes. Greater than 10 percent of these transcriptional regulators showed differential levels of expression across the time points, falling into various clusters or trends of expression. We observed a burst of transcription at 24hrs in both organs, but could discern differences between utricle and cochlea regeneration including changes in CEBPG, ETV1 and POU5F. We also observed commonalities in both organs, including upregulation of HES2, HSF1, and ERF. The laser ablation time course exhibited a reproducible burst of gene expression at 1hr followed by a second cycle of increased expression at 3hrs including HOXA13, and RORC. Identifying key transcriptional regulators in these processes should provide insights into the genetic programs necessary for hair cell regeneration in avians, and may provide clues to the differences between these mechanisms and those in mammals.

**Gene expression profiles of FAC sorted mouse inner ear cells.** S.A. Sajan<sup>1</sup>, R. Veile<sup>1</sup>, P. White<sup>2</sup>, A. Doetzlhofer<sup>2</sup>, A. Groves<sup>2</sup>, N. Segil<sup>2</sup>, M. Lovett<sup>1</sup>. 1) Genetics, Washington University, St. Louis, MO; 2) House Ear Institute, Los Angeles, CA.

Late onset hearing loss affects almost 30 million Americans and is primarily due to irreversible damage to the inner ear auditory sensory epithelium. This epithelium consists of hair cells and surrounding supporting cells. Hair cells are mechanoreceptors that initiate action potentials in response to sound (or in the case of the vestibular organ, movement). One route towards restoring auditory hair cell function is cell replacement therapy. This would require *in-vitro* hair cell differentiation. To decipher the genetic programming of mammalian hair cell development we used a human transcription factor custom microarray and created gene expression profiles of various purified cell types from the mouse inner ear. The mouse atonal homolog1 (*Math1*) and cyclin-dependent kinase inhibitor *p27<sup>Kip1</sup>* are two of the few known markers of hair cells and supporting cells, respectively. We obtained FAC-sorted cochlear and vestibular hair cells at post natal days 1-3 from a transgenic mouse strain expressing *GFP* downstream of the *Math1* regulatory elements. Cochlear supporting cells from the same stage were obtained from mice expressing *GFP* downstream of the *p27<sup>Kip1</sup>* regulatory elements. Unlabeled cochlear and vestibular cells were also obtained from these mice. Several pairwise microarray comparisons were conducted on these various cell populations. The microarray data-sets were analyzed, and lists compiled of differentially expressed genes. Over 60 TF genes were over-expressed in hair cells and approximately 50 genes were over-expressed in supporting cells. We are currently validating this data-set by quantitative-PCR, in-situ hybridizations and RNA-interference experiments. These observations provide new insights into the developmental programming of these important cell types, as well as potential new markers for lineage tracing. Finally, we have also constructed cDNA libraries from these sorted cell populations. These cDNA collections from purified cell types should be valuable resources for future EST analyses.

**Expression pattern of the gene responsible for Dyggve-Melchior-Clausen syndrome (*Dym*) during human development.** V. El Ghouzzi, G. Mattei, V. Paupe, T. Attie-Bitach, A. Munnich, M. Vekemans, V. Cormier-Daire. Department of Medical Genetics and INSERM U393, Recherches sur les Handicaps Génétiques de l'Enfant, Hôpital Necker-Enfants Malades, 149, rue de Sèvres, 75015 Paris, France. elghouzzi@necker.fr or cormier-daire@necker.fr.

Dyggve-Melchior-Clausen syndrome (DMC) is a rare autosomal recessive skeletal disorder characterized by spondylo-epi-metaphyseal dysplasia and mental retardation. Studying DMC, we have recently identified nonsense, frameshift and splice mutations in a novel evolutionarily conserved gene, *Dym* (*FLJ20071/FLJ90130*), which encodes Dymeclin, a 669 amino-acid product of unknown function. Electron microscopy analysis on skin biopsy from DMC patients strongly suggests a storage disease as the cytoplasm of all cells displayed an enlarged endoplasmic reticulum and a large number of membranous vacuoles containing amorphous material. However, the accumulated metabolite remained unidentified. In order to determine the expression pattern of *Dym* during human development, RT-PCR analyses, multiple tissue expression arrays and *in situ* hybridizations were performed on several human embryo-fetal and adult tissues. We found that *Dym* is widely expressed but particularly in germinative zones of brain, lung, gut epithelium, limb buds and orofacial structures of 7 and 8 weeks human embryos. Interestingly, *Dym* transcripts were not detected in bone and cartilage at these stages but later, in post-natal chondrocytes, osteoblasts and fibroblasts. In addition, RT-PCR experiments and Northern blot hybridizations indicated that the mutated transcripts were not or weakly expressed in patients fibroblasts, suggesting that a specific mRNA decay occurs in DMC. These data indicate that DMC results from a loss-of-function of *Dym* at the transcriptional level, which would trigger storage of an unidentified substrate and suggest that *Dym* exert a role in early brain patterning, but may act later in the growth plate. This work was supported by the European Skeletal Dysplasia Network (ESDN).

**Disruption of the gene encoding for E-Selectin Ligand 1 (ESL-1) in mouse causes a reduction of skeletal growth independent of the FGFR signaling.** *R. Mendoza-Londono*<sup>1</sup>, *K. Li*<sup>2</sup>, *M. Jiang*<sup>1</sup>, *H. Lu*<sup>3</sup>, *J. Hicks*<sup>4</sup>, *A. Beaudet*<sup>1</sup>, *B. Lee*<sup>1,5</sup>. 1) Depts. of Molecular and Human Genetics; 2) Surgery; 3) Medicine; 4) Pathology and; 5) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX.

E-Selectin ligand 1 (ESL-1) is expressed on the cell surface of multiple cell lines. It is highly homologous to the chicken cysteine-rich fibroblast growth factor receptor (CFR) and the rat and human golgi apparatus protein 1 (MG160 and GLG1). ESL-1 was first isolated based on its ability to bind E-selectin, but its role as a resident Golgi apparatus protein is still unclear. ESL-1 binds fibroblast growth factors (FGF) with high affinity, but unlike FGF receptors, it lacks the intracytoplasmatic tyrosine kinase domain required for signaling. Because of its homology to CFR, we asked whether ESL-1 regulates FGF signaling during development. We have generated an ESL-1 null mouse by targeted disruption of the ESL-1 locus. ESL-1 heterozygous mice are phenotypically normal. The homozygous mutant mice are viable but only 65% survive to weaning. Mutant mice are 30% smaller than their wildtype littermates and skeletal preparations show generalized shortening and thinning of the long bones, with no patterning defect. X-rays of the skeleton show shortening of all bony elements with an apparent decrease in bone mineral density. Histological analysis demonstrated shortening and minimal disorganization of the growth plates, with fewer cells in the proliferating and hypertrophic zones and moderate increase in bone trabeculae thickness. Immunohistochemical analyses for Stat-1, Stat5a and p21, which are downstream targets in the FGFR/Stat-1 pathway, did not show differences between (+/+) and (-/-) animals. To study the role of FGFR3 signaling in the ESL-1-null mice we generated ESL-1 (-/-) /FGFR3(-/-) double mutants. These mice did not show correction of the skeletal phenotype, further suggesting that ESL-1 acts independently of FGFR3 signaling. These in vivo data suggest that ESL1 regulates chondrogenesis in a pathway autonomous of FGF signaling and is a candidate gene for skeletal dysplasias with similar chondro-osseous features.

**A search for causative genes of split hand/foot malformation (SHFM3).** *H. Kano*<sup>1</sup>, *H. Kurahashi*<sup>1, 2</sup>, *T. Toda*<sup>1</sup>. 1) Division of Functional Genomics, Department of Post-Genomics and Diseases, Osaka University Graduate School of Medicine, Osaka, Japan; 2) Division of Molecular Genetics, Institute for Comprehensive Medical Science, Fujita Health University, Aichi, Japan.

Split hand/foot malformation (SHFM) is a limb malformation involving the central rays of the autopod and presenting with syndactyly and median clefts of the hands and feet. At least five loci for SHFM have been mapped. The Dactylaplasia (Dac) mouse is considered a model for human SHFM3 as the human 10q24 region is homologous to the Dac locus on mouse chromosome 19. Two independent Dac mutations in the mouse dactylin gene have been identified. Both mutations found in Dac mice were insertions. We first cloned these insertions of 7,486 bp for Dac1 and 7,473 bp for Dac2, respectively. The insertions were LTR transposons called ETn and they were 99.6% identical. However, one of the Dac alleles has been shown neither to affect the amount nor the size of the dactylin transcript. In addition, no mutations in the human dactylin gene have been reported. Therefore, these Dac alleles may cause long-range disruption of the regulation of one or more other genes, leading to the pathogenesis of Dac mice. With the aim to elucidate this hypothesis, we investigated the expression levels of genes mapped to the homologous region of human SHFM3-candidate locus. However, genes in the Krd deletion were excluded from the analysis, because the Krd mouse has no limb abnormality despite a deletion encompassing 5 cM on mouse chromosome 19. Hence, the remaining region which was 1 Mb long, including 30 transcripts was analyzed. We used quantitative real-time RT-PCR to measure the expression levels of these transcripts in Dac embryos at various stages. Results were normalized to -actin and compared to their expression levels in wild type embryos. Although most transcripts were expressed normally in Dac embryos, a few transcripts exhibited lower levels of expression. We are now searching for mutations in these genes, in 32 unrelated SHFM patients. To date no mutation has been found, however, we expect that the causative gene of SHFM3 is amongst these genes.

**Expression of Fibroblast Growth Factor Receptor 3 (FGFR3) in glial cells of a TDI fetus carrying a K650M mutation.** *L. Legeai-Mallet<sup>1</sup>, P. Loget<sup>2</sup>, J. Martinovic<sup>1</sup>, S. Heuertz<sup>1</sup>, C. Benoist-Lasselin<sup>1</sup>, A. Munnich<sup>1</sup>, J. Bonaventure<sup>1</sup>, F. Encha-Razavi<sup>1</sup>.* 1) Génétique INSERM U393, Hôpital Necker, Paris, France; 2) Service d'anatomopathologie CH Le Mans, France.

Thanatophoric Dysplasia (TD) is a neonatal lethal skeletal dysplasia caused by common FGFR3 mutations in different domains of the protein. Based on radiological, histological and molecular criteria, TD has been divided into type 1 (TDI) and type 2 (TDII). While TDII is caused by a single K650E mutation in the tyrosine kinase 2 (TK2) domain of the receptor, conversion of the same lysine residue into methionine (K650M) can produce either TDI or Severe Achondroplasia with Developmental Delay and Acanthosis Nigricans (SADDAN). Here we identified a K650M FGFR3 mutation in a 24-week fetus presenting with typical TDI features. Clinical and radiological analyses revealed that the fetus had rhizomelic shortening of the limbs, curved femora, platyspondyly and narrow thorax. At the histological level the femoral growth plate had reduced size, the chondrocyte columns were totally absent and the line of ossification was distorted. In keeping with previous studies, high level of the FGFR3 protein was found in the pre-hypertrophic zone of the growth-plate. Immunostaining showed an abnormally high amount of STAT1, STAT3 and STAT5 proteins in the pre-hypertrophic cells confirming constitutive activation of the receptor. Brain examination showed macrocephaly and classical abnormalities of the temporal lobes associated with severe hydrocephalus due to third ventricle atresia. Interestingly FGFR3 expression in the brain was restricted to glial components (ependymal periventricular cells and astrocytes). Comparison with an age-matched control showed a slightly higher expression of the receptor in the ependymal cells of the TDI fetus whereas neurons of the cortical plate and deep brain nuclei were negative. Since glial cells are involved in neuronal migration, our findings raise the question of whether abnormal gyration of the temporal lobes in TDI is directly related to defective neuronal migration. This work was supported by the European Skeletal Dysplasia Network (ESDN).

Program Nr: 891 from 2003 ASHG Annual Meeting

**Molecular and Biochemical analysis of Oligophrenin, a RhoGAP, in Fibroblasts.** *A.L. Gropman, B.W. Howell.*  
Neurogenetics Branch National Institutes of Health 10 Center Drive Building 10, Bethesda MD 20892.

Recently, interest has been generated in the members of the Rho GTPases which play a role in actin cytoskeleton dynamics. These small proteins have been shown to influence cell shape, migration, adhesion, and connectivity via regulation of actin stress fiber formation and extension of lamellipodia and filopodia. Several Rho family members and their effectors have been identified and believed to play a role in mental retardation, cancer progression, and neurodegenerative disorders. The oligophrenin gene, encoding a RhoGAP, has recently been implicated in X-linked mental retardation with associated cerebellar hypoplasia and seizures. It is up-regulated in several. Oligophrenin contains a pleksin homology domain, a RhoGAP domain, and is highly conserved between human and mouse; its function remains unknown. In order to investigate the function of oligophrenin as a potential mediator of neuronal migration/cytoskeletal changes, we performed a number of in vitro experiments. Using an antibody to the C-terminus of oligophrenin, we have observed oligophrenin expression in E16 cortical neurons with a diffuse cytoplasmic distribution. Histologic examination of E15 fetal mouse brain shows diffuse immunoreactivity. In adult tissue sections, expression is high in the rostral migratory stream, entorhinal cortex, septal area, hippocampus, and dentate gyrus. In transfection experiments, we have observed that fibroblast cell lines overexpressing oligophrenin have very few stress fibers, consistent with a role for oligophrenin in the suppression of Rho GTP levels. In yeast two hybrid screens, we have identified a panel of potential interactors. In vitro association studies with one candidate, VACM-1 (Vasopressin activated calcium mobilizing receptor-1), which is homologous with a member of the cullin family of proteins implicated in the regulation of cell cycle through the ubiquitin-mediated degradation of cyclin-dependent kinase inhibitors shows a positive association in co-immunoprecipitation studies. This suggests that oligophrenin may participate in a signaling pathway involved in ubiquitin mediated degradation.

***mage*: a MAGE/NDN-like gene in zebrafish.** *J.M. Bischof*<sup>1</sup>, *M. Ekker*<sup>2</sup>, *R. Wevrick*<sup>1</sup>. 1) Department of Medical Genetics, University of Alberta, Edmonton, Alberta, Canada; 2) Ottawa Health Research Institute and Department of Medicine, University of Ottawa, Canada.

The human necdin/MAGE gene family has over 50 members identified, but most of the proteins encoded by these genes are of unknown function. Two gene family members on chromosome 15q11, *NDN* (necdin) and *MAGEL2*, are inactivated in the neurobehavioral gene deletion syndrome Prader Willi Syndrome. *NRAGE/MAGED1* is an X-linked member of the same gene family that is implicated in neurotrophin signaling. We have now identified a single locus in *Danio rerio* that encodes a putative protein with significant coding sequence similarity the MAGE homology domain, a 165-171 amino acid motif of shared sequence similarity among members of the mammalian NDN/MAGE gene family. Highest similarity was found to *Mageg1* and *Maged1*, and like *Maged1*, zebrafish *mage* is multi-exonic within the open reading frame. The 1072 nucleotide cDNA has a 260 amino acid open reading frame and is represented by UniGene Dr.2185, which we placed at a single map position near the top of LG23. Analysis of the complete *Fugu ribripes* genome sequence also gives evidence for the existence of only a single MAGE-like gene in teleost fish. We show that *mage* is expressed in several regions of the larval and adult brain, specifically the retina, the medial region of the telencephalon, periventricular gray zone of the optic tectum, and most highly in the cerebellar corpus. Specific down-regulation of *mage* translation in early development using a morpholino oligonucleotide had no effect on embryonic development. At present there are no induced or naturally occurring zebrafish mutations genetically mapped in proximity to the location on LG23 containing *mage*. The identification and characterization of such a mutant and further investigation of the role of these proteins in neural development and function is important to resolve issues of parallel functions of MAGE genes in vertebrate species.

**Establishment of a tetracycline inducible system for the ectopic expression of Zic2 during brain development.** *S. Brown, L.Y. Brown.* Ob/Gyn, Columbia University, New York, NY.

Heterozygous null mutations in the transcription factor gene ZIC2 have been previously shown to result in holoprosencephaly (HPE) in humans, while diminished levels of the Zic2 protein cause HPE in mice. Thus, brain development is exquisitely sensitive to the level of Zic2 protein. Despite studies in several model systems, there is currently little understanding of how Zic2 functions during brain development. We have hypothesized that mis-expression of Zic2 during development may be a good system for attempting to understand Zic2 function, and to this end, we have used transient transgenic mice to mis-express Zic2 during brain development. Zic2 mis-expression indeed results in severe brain malformations, but for this reason, it is impossible to establish a stable transgenic line and detailed studies of how Zic2 acts to cause malformation have not been possible.

To circumvent this problem, we have used the tetracycline regulated system to establish a modular, inducible system for over-expressing and mis-expressing Zic2 during development in mice. In this system, a stable line harboring a Zic2 transgene under the regulation of the tetracycline response element (TRE) can be crossed with other transgenic lines that express the reverse tetracycline transactivator protein (rtTA). Maternal administration of tetracycline then results in expression of transgenic Zic2 in compound heterozygote embryos. Thus, transgene expression can be regulated with respect to location, developmental time and level.

We have developed TRE-Zic2 mouse lines and we have performed crosses between these and several rtTA expressing lines. These studies show the feasibility of this system and have allowed us to produce embryos with tetracycline inducible brain malformations. Studies of how ectopic expression of Zic2 alters the expression of other developmental genes and leads to malformation are ongoing and promise to lead to important insights into the role of Zic2 in brain development.

**Imprinting and the brain: Maternal influences on cognitive skills.** *L. Goos, I. Silverman.* Dept of Psychology, York Univ, Toronto, ON, Canada.

This study represents one of the first attempts to investigate the influence of genomic imprinting on normal brain development and adult behaviour. Animal experimentation and clinical studies have indicated that the maternal and paternal genomes play differential roles in the development of the mammalian brain. In particular, evidence suggests that the maternal genome makes a disproportionate contribution to the development of the neocortex, with increasing influence from the occipital to the frontal lobes. Numerous psychological and psychophysical tasks have been designed and validated for cortical localization of function and dysfunction in normal and clinical populations. Appropriate tasks were utilized in an intra-familial design to determine if parental influence on cognitive task performance could be discriminated. It was expected that children's scores would become more like their mothers' scores and less like their fathers' scores as the cortical region recruited by the task changed from the occipital to the frontal lobes. 65 biological families were recruited as participants, with members ranging in age from 17-70. Where significant correlations between parent and child scores exist, the predicted trend is obtained eight times out of twelve. Mother-child correlations were higher than father-child correlations in 12 out of 16 comparisons (75%), a frequency unlikely to occur by chance (binomial expansion,  $p=0.03$ ). Stepwise, hierarchical regression showed that maternal score was the sole predictor for mean child score for frontal, parietal and temporal tasks ( $F$ 's (1,63)=20.225, 12.632, 10.156 respectively,  $p$ 's 0.01). Paternal score for these locations did not account for any further reductions in variance. Paternal score was a significant predictor of mean child score for the occipital lobe tasks ( $F(1, 63)=15.276$ ,  $p<0.000$ ), with the influence of mothers approaching significance ( $F(2, 62)=3.63$ ,  $p=0.06$ ).

**Developmental instability and isolated sagittal craniosynostosis.** *K. Aldridge<sup>1</sup>, V.B. DeLeon<sup>2</sup>, J.T. Richtsmeier<sup>1</sup>.* 1) Department of Anthropology, Pennsylvania State University, University Park, PA; 2) Center for Functional Anatomy and Evolution, Johns Hopkins University School of Medicine, Baltimore, MD.

Developmental instability is defined as noise or disruption in the normal pathway of development. Previous research has demonstrated characteristic dysmorphology in the skulls and brains of children with isolated sagittal craniosynostosis. The purpose of this study is to determine whether these dysmorphologies are associated with developmental instability, as determined by the degree of fluctuating asymmetry in the skeletal and central nervous systems. We predict that perturbations in growth associated with the synostosed suture increase levels of fluctuating asymmetry.

A sample of children with premature midline fusion of the sagittal suture was compared to an age-matched sample of morphologically normal children. Three-dimensional landmark coordinate data from the skull and brain were collected using computer reconstructions of CT and MRI data. An application of Euclidean Distance Matrix Analysis (EDMA) was used to quantify and compare the degree of fluctuating asymmetry in the two groups.

Comparisons of fluctuating asymmetry levels in the brain produced inconclusive results, with the relative degree of asymmetry varying across different regions of the brain. Contrary to our prediction, the degree of fluctuating asymmetry in the skulls of individuals with craniosynostosis is less than that of the normal control sample, indicating a more stable developmental system in craniosynostosis. The differential effects of the constrained suture on developmental instability in the skeletal and nervous systems may be related to the sequential occurrence of developmental events in these systems.

This research was supported by NIDCR grant 1 P60 DE13078 (Project VI, PI: JTR).

**Good Babies Sleeping Poorly: Insufficient Sleep in Infants with Smith-Magenis Syndrome (SMS).** *W.C. Duncan*<sup>1</sup>, *A. Gropman*<sup>2</sup>, *R. Morse*<sup>3</sup>, *D. Krasnewich*<sup>3</sup>, *A.C.M. Smith*<sup>3</sup>. 1) NIMH; 2) NINDS; 3) MGB/NHGRI, NIH, HHS, Bethesda, MD.

Sleep disturbance in Smith-Magenis syndrome (SMS) has been described in early childhood through adulthood. To our knowledge there have not been any descriptions of sleep quality in SMS infants (1 year). While maladaptive behavior accompanies the sleep disturbance in young children with SMS (Dykens & Smith, 1998), happy complacency is often reported of SMS infants. The sleep disturbance in young children with SMS is partly attributed to deficient nighttime levels, and elevated daytime levels of melatonin. While many parents do not report unusual sleep patterns in undiagnosed SMS infants, anecdotal reports have suggested the possibility of sleep disturbance in this infant SMS population.

Actigraphy, a non-invasive technique used to measure rest and activity, was used to estimate home sleep behavior in twelve children with confirmed SMS. Four age groups were studied: infants (1y, n=3), preschool (3y, n=3), early school (5y, n=3) and later school (6-8y, n=3). All children were enrolled in the IRB-approved NIH natural history study and continued on their existing drug regimen during the study. All subjects wore a wrist or ankle Actiwatch for 2-4 weeks to quantify rest/activity patterns. Data were analyzed using Actiware-Sleep software (Mini Mitter Co, Inc.). Sleep estimates were derived for 24 hours (24H), daytime (L) and nighttime (N) and compared with previously reported values. Actigraphy-estimated sleep was reduced in the infant SMS group (24H=11.05 h; L=3.00 h; N=8.05h). Consistent with prior reports, estimated 24 hour and night time sleep in the three older age groups were also reduced: preschool (24H=9.5h, N=8.5h), early school (24H=8.6h, N=7.3h) and later school (24H=7.8h, N=7.5h). These results indicate the presence of a phenotypic sleep disturbance in SMS infancy, and document a progressive decline in estimated sleep from infancy to eight years.

**Dynamic expression and localization of MeCP2 in the developing mouse brain.** *J.M. LaSalle, D. Braunschweig, T. Simcox, R.C. Samaco.* Med Micro and Immuno and Rowe Program in Human Genetics, UC Davis Sch Med, Davis, CA.

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder caused by mutations in *MECP2*, encoding the methyl-CpG-binding protein 2 (MeCP2). Despite ubiquitous expression of *MECP2*, the onset of symptoms in RTT is delayed until 6-18 months and limited to the CNS. MeCP2 protein expression shows cellular heterogeneity in the CNS and elevated MeCP2 expression is acquired in individual neurons during postnatal development. *Mecp2*-null mutations targeted to postmitotic neurons exhibit a similar phenotype as germline *Mecp2* mouse mutants. These combined results suggest that *MECP2* mutations are primarily manifested in mature MeCP2<sup>hi</sup> neurons. The onset of symptoms in the mouse is delayed until 3-6 weeks in males and 4-6 months in females, developmentally later than expected from female RTT patients. To further understand the developmental ontogeny of MeCP2, we have used laser scanning cytometry to perform quantitative localization of MeCP2 in individual cells within sagittal mouse brain sections during embryonic and postnatal development. While virtually all cells (>90%) were positive for MeCP2, heterogeneity in the level of expression was observed between different brain regions, developmental stages, and cellular subpopulations, resulting in >10-fold increases from embryonic (E10) to a 40-wk adult. Increases in MeCP2 expression were not completely linear throughout development as had been predicted from humans, but instead showed dynamic changes from embryonic to 3-wk postnatal. High MeCP2 expression in the cerebral cortex from 3-6 wks corresponded with the observance of a RTT phenotype in the *Mecp2*-null males. From 7-40 wks there was a positive correlation with age in the mean MeCP2 expression due to an increase in the MeCP2<sup>hi</sup> population from 31% to 47%. The continued increase in the %MeCP2<sup>hi</sup> cerebral neurons during normal mouse adulthood may help explain the delay of symptoms in heterozygous female mice. In conclusion, these results demonstrate dynamic developmental regulation of MeCP2 and provide support to the hypothesis that *MECP2* mutations are primarily manifested in the MeCP2<sup>hi</sup> population that emerges progressively during postnatal brain development.

**A microarray screen for genes involved in brain regionalization and morphogenesis.** Z. Luo<sup>1, 2</sup>, D. Tentler<sup>1, 2</sup>, L. Kudo<sup>3</sup>, C. Sabatti<sup>4</sup>, H.I. Kornblum<sup>5</sup>, D.H. Geschwind<sup>1,2</sup>. 1) Dept. of Neurology; 2) Program in Neurogenetics; 3) Neuroscience IDP; 4) Dept. of Human Genetics; 5) Dept. of Molecular & Medical Pharmacology, UCLA School of Medicine, Los Angeles, CA.

A large number of genes responsible for human neurodevelopmental disorders are likely to be also involved in brain patterning and regionalization. For example, the sonic hedgehog gene is involved in autosomal dominant holoprosencephaly type 3 and the paired box gene-6, which is responsible for aniridia type II, are known to play a key role in establishing of brain organization and morphology. We performed a large-scale microarray study in order to identify genes expressed in medial lateral gradients during the early stages of cerebral cortical regionalization, and therefore likely to be involved in cerebral axis formation and brain patterning. Messenger RNA was extracted separately from the left, right, and medial parts of mouse E10.5 telencephalon and Representational Differences Analysis (RDA) subtraction followed by microarray screening was performed, similar to previously published methods (Geschwind et al., Neuron 2001, 29: 325-339). We identified 33 known and 6 ESTs enriched in the medial cortical anlage and 80 known and 19 ESTs enriched in the lateral region. We confirmed differential expression of some known and novel genes by whole-mount in situ hybridization and by comparison with published literature. Gene ontology analysis and pathway analysis was performed to further categorize genes. A number of genes already known to be involved in human neurodevelopmental and neurodegenerative disorders, such as ZIC-3 and the multiple exostosis type 1 gene were identified. The chromosomal location of the known and unknown genes was determined to further investigate the candidacy of these brain patterning genes for producing mental retardation or other neurodevelopmental phenotypes.

**Tissue specific FGFR3 transgenic mouse models for Thanatophoric Dysplasia Type II.** *T. Lin*<sup>1</sup>, *S.B. Sandusky*<sup>1</sup>, *H. Xue*<sup>2</sup>, *K. Fishbein*<sup>3</sup>, *R. Spencer*<sup>3</sup>, *M. Rao*<sup>2</sup>, *C.A. Francomano*<sup>1</sup>. 1) Laboratory of Genetics, NIA, NIH, Baltimore, MD; 2) Laboratory of Clinical Investigation, NIA, NIH, Baltimore, MD; 3) Laboratory of Neurosciences, NIA, NIH, Baltimore, MD.

Neonates with thanatophoric dysplasia have profound skeletal manifestations and several reported CNS alterations as a result of mutations in fibroblast growth factor 3 (FGFR3). To investigate the effect of the FGFR3 K644E mutation on central nervous system (CNS) development, we have generated tissue specific thanatophoric dysplasia type II mice (TDII) by crossing *Fgfr3*<sup>+/*neo*-K644E</sup> transgenic mice with either CNS specific (Nestin-cre) or cartilage specific (Col2a1-cre) mice. TDII/Nestin-cre (TDII-N) neonates did not demonstrate a profound skeletal phenotype compared to TDII/Col2a1-cre (TDII-C). TDII-N pups were comparable to their wild type littermates in terms of tail length, fore and hindlimbs, and body weight, however, many pups did exhibit notably round heads. MRI and histochemical analysis illustrated asymmetrical changes in cortical thickness and cerebellar abnormalities in TDII-N mice, which correlate with brain abnormalities observed in human TDII patients. Such abnormalities were not seen in TDII-C mice. Overall, these data indicate that the tissue specific mouse model is an excellent system for studying the role of FGFR3 in the developing CNS and skeleton.

**Expression analysis of the LMX1B gene supports expansion of the Nail Patella Syndrome phenotype. I.**

*McIntosh<sup>1</sup>, J.A. Dunston<sup>1</sup>, T. Reimschisel<sup>1</sup>, R.L. Johnson<sup>2</sup>, E. Sweeney<sup>3</sup>.* 1) Inst Genetics Medicine, Johns Hopkins Medical Sch, Baltimore, MD; 2) Dept. of Biochemistry & Molecular Biology, M.D. Anderson Cancer center, Houston, TX; 3) Clinical Genetics, Royal Liverpool Childrens Hospital, Alder Hey, UK.

LMX1B is a member of the LIM-homeodomain family of transcription factors. Heterozygous loss-of-function mutations in LMX1B cause Nail Patella Syndrome (NPS). NPS is a pleiotropic phenotype, classically regarded as a connective tissue disorder, in which the cardinal features include dysplasia of the nails and hypoplasia/dysplasia of the patellae and elbow joints. Nephropathy and glaucoma have also been recognized as part of the syndrome. In parallel with transgenic mouse experiments to determine the elements responsible for the regulation of LMX1B expression during development, we have undertaken an analysis of the expression of the endogenous *lmx1b* gene. A lacZ reporter gene was inserted into the 3-UTR of the murine *lmx1b* gene via site-specific recombination in ES cells. Expression of *lmx1b* was monitored at E9.5 to E15.5 by -galactosidase staining. The expression of *lmx1b* in the dorsal limb mesenchyme, kidney and anterior chamber of the eye have been reported previously, and explain the defects in these structures/organs observed in NPS patients. LMX1B is unusual amongst members of the LIM-homeodomain family in being expressed abundantly outwith the CNS. Expression within the midbrain has also been reported (1) and *Lmx1b* expression has been used as a marker for the differentiation of dorsal interneurons within the spinal cord (e.g. ref. 2). We have confirmed these observations and also noted expression in the ventral floor plate and the primordial mammary glands, vibrissae and hair follicles. The CNS expression of *lmx1b* may explain the symptoms of Attention Deficit (Hyperactivity) Disorder (ADHD), peripheral neuropathy, Reynauds phenomenon and epilepsy observed in a large cohort of NPS patients (3).

Refs: (1) Smidt et al, Nat Neurosci 3:337, 2000. (2) Müller et al, Neuron 34:551, 2002. (3) Sweeney et al, J Med Genet 39:152, 2003.

**Abnormal expression of MAP1B in *fmr1* ko neurons during early neuritogenesis.** *R.R. Lu<sup>1</sup>, W. Li<sup>2</sup>, L. Ku<sup>2</sup>, H. Wang<sup>2</sup>, Y. Feng<sup>2</sup>.* 1) Medicine, Keck School of Medicine at USC, South Pasadena, CA; 2) Pharmacology, Emory University, Atlanta, GA.

Fragile X mental retardation results from the lack of FMRP, a selective RNA binding protein implicated in regulating protein synthesis by interaction with its bound mRNAs. More than 400 FMRP associated mRNAs in the adult mouse brain have been identified recently using microarray approaches. One mRNA ligand on the microarray showed sequence homology to the microtubule-associated proteins (MAPs) 1A and 1B, raising a hypothesis that FMRP may be involved in regulating microtubule stability via controlling translation of MAP1A/B, which in turn governs normal neuritogenesis and synapse development. Consistent with this hypothesis, delayed dendritic spine maturation has been reported in fragile X patients as well as in *fmr1* knockout mice. We directly tested the above hypothesis by examining FMRP's influence on MAP1A/B in early neuritogenesis, since the most severe abnormality observed in the neonatal brain of the *fmr1* knockout mice. We found that both MAP1A and MAP1B mRNAs were associated with FMRP in young mouse brain, with much higher quantity of MAP1B than MAP1A in immunoprecipitated FMRP complexes. Immunoblot analysis further revealed elevated MAP1B protein level in the developing hippocampus and early stages of primary cultures of cortical neurons derived from the *fmr1* knockout brain without increased MAP1B mRNA expression. Finally, linear sucrose gradient analysis indicated abnormally increased translation of MAP1B in the *fmr1* knockout neurons. These results support our hypothesis that FMRP is a translation suppressor that accurately regulates MAP1B expression during the early development of brain neurons. The lack of FMRP leads to elevated MAP1B, which in turn results in misregulation of microtubule dynamics in neuritogenesis as well as synapse maturation.

**Type III lissencephaly: a spectrum of neuro-ectodermal dysplasia?** *J. Martinovic<sup>1</sup>, F. Francis<sup>2</sup>, A. Benachi<sup>3</sup>, J. Roume<sup>4</sup>, M. Gonzales<sup>4</sup>, C. Fallet<sup>5</sup>, M. Vekemans<sup>1</sup>, J. Chelly<sup>2</sup>, F. Encha-Razavi<sup>1</sup>*. 1) Unit of Embryo-Fetal Development, Hosp Necker - Enfants Malades, Paris, France; 2) INSERM U129, Institut Cochin de Genetique Moleculaire; 3) Unit of Fetal Medicine, Hopital Necker-Enfants Malades; 4) Unit of Fetal Pathology, Hopital Saint-Antoine; 5) Dept. of Neuropathology, Hopital Saint-Anne, Paris, France.

Lissencephaly is defined by the absence or rarefaction of cerebral convolutions. Two major groups, type I and II are classically described. A third type (LIS III) with distinct neuropathological pattern was first reported in Neu-Laxova syndrome (NLS), and recently in an apparently different context of Fetal Akinesia Deformation Sequence (FADS) (OMIM N601160) with thick and/or fragile skin, but no ichthyosis. The purpose of this study is to describe a comparable family with four affected and two unaffected sibs in which the phenotypically healthy parents were first cousins of Turkish origin. Four affected fetuses terminated at 24, 18, 26, and 24 weeks respectively, presented severe growth delay with microcephaly, limb contractures with pterygia, and syndactyly. All presented microcephaly/lissencephaly, delayed cerebral cortex maturation, lack of callosal and cerebrospinal tracts, neuronal depletion of the germinal zones, basal ganglia, brain stem nuclei, and spinal cord. Viscreal exam showed bilateral adrenal agenesis. Thick and fragile skin without ichthyosis was noticed in all fetuses affected. Genetic analysis using homozygosity mapping was carried out with microsatellite markers evenly distributed over the whole genome. Homozygosity was observed for several regions of the genome. Currently, we are testing new set of markers for fine mapping, and assessing non excluded regions because of the non informativity of markers in the initial panel. Our hypothesis is that LIS III might be the common denominator in the neuroectodermal dysplasia spectrum including NLS and OMIM N 601160. The identification of the gene responsible for these disorders may shed further light on the frontier between these two entities, and possibility of a lesional continuum between them.

**Multiple pathways regulate MeCP2 in normal brain development and exhibit defects in non-*MECP2* mutant Rett and autism samples.** *R.C. Samaco, R.P. Nagarajan, D. Braunschweig, J.M. LaSalle.* Med Micro & Imm, UC Davis Sch Med, Davis, CA.

Mutations in *MECP2* cause Rett syndrome (RTT) but have also been found in other neurodevelopmental disorders, suggesting an important role for MeCP2 in normal brain development. Although *MECP2* is ubiquitously transcribed, MeCP2 protein expression is complex and developmentally regulated, involving alternatively polyadenylated transcripts and heterogeneous expression in the postnatal CNS. We hypothesized that MeCP2 expression changes may be defective in RTT, autism, and potentially other neurodevelopmental disorders that do not exhibit mutations in *MECP2*. We therefore performed a high-throughput analysis of MeCP2 expression on a tissue microarray containing 28 brain samples from RTT, autism, AS, PWS, PDD, and age-matched controls. Combined analyses of MeCP2 immunofluorescence plus FISH using riboprobes for the *MECP2* coding region (CDS) and alternative long transcript (3UTR) were performed. Negative controls included nonspecific IgG and sense strands of CDS and 3UTR riboprobes, while histone H1 and actin antisense controlled for variability of human brains. Combined quantitative analyses of MeCP2 protein and RNA were performed by laser scanning cytometry and tested for significant differences from age-matched controls. Normal cerebral samples showed an increase in total MeCP2 expression and %MeCP2<sup>hi</sup> cells and a decreased ratio of 3UTR to total CDS with age. The MeCP2<sup>hi</sup> cells had significantly higher CDS and 3UTR transcription, suggesting that the developmental switch in *MECP2* transcript usage is independent from the transcriptional increase that regulates the MeCP2<sup>lo</sup> to MeCP2<sup>hi</sup> transition. Several autism samples showed significant defects in MeCP2 protein levels, apparently by different transcriptional and posttranscriptional mechanisms. In contrast, the 3 RTT samples with *MECP2* mutations showed defects in MeCP2, but not CDS or 3UTR/CDS ratio. A RTT sample without a detectable *MECP2* mutation was deficient in the %MeCP2<sup>hi</sup> cells and the 3UTR/CDS ratio within the MeCP2<sup>hi</sup> population. These results suggest that defects in multiple pathways that regulate the complex developmental expression of MeCP2 may contribute to RTT and other autism-spectrum disorders.

**Sexually dimorphic expression of an X-linked deubiquitinating protease in mouse brain.** *J. Xu, A.P. Arnold.* Dept Physiological Sci, Univ California, Los Angeles, Los Angeles, CA.

Sexual differentiation of the brain and behavior is thought to be due largely to the direct action of gonadal steroid hormones on the brain. Recent evidence, however, indicates that some sex differences in brain cannot be easily explained as the result of steroid action. We hypothesize that genes on the sex chromosomes are expressed in the brain and cause sex-specific patterns of development. The X chromosome encodes a large number of genes essential for brain development. The dosage difference between males and females in expression of X genes is not entirely equalized by X-inactivation. Some X genes escape X-inactivation and X-inactivation may be a function of age and/or tissue type, therefore females may express specific X genes more highly than males do. We measured the expression of *Usp9x*, an X gene encoding a ubiquitin specific protease which regulates proteasome-mediated protein degradation. *Usp9x* has been found to be involved in neuronal differentiation and synaptic plasticity. Using northern and western blot analysis, we found *Usp9x* mRNA and protein are expressed higher in the whole brain of adult female mice than in males. *Usp9x* mRNA is also expressed higher in XX males and XX females than in XY males and XY females (sex defined by gonadal phenotype), therefore the sex difference in *Usp9x* expression is likely related to sex chromosome complement. The expression of Y homologous *Usp9y* is low or absent in adult brain and therefore cannot balance the sex difference in *Usp9x*. With immunohistology we localized *Usp9x* protein to cerebral cortex, hippocampus and cerebellum. In hippocampus *Usp9x* is found to be higher in CA3 than in CA1 or dentate gyrus. Cerebellar Purkinje cells *Usp9x* are labeled in a patchy pattern which is parallel to that of  $\beta$ -catenin, a putative substrate for *Usp9x* deubiquitinating activity. The sex difference in *Usp9x* expression suggests that sexually dimorphic gene expression could arise as a consequence of the complement of sex chromosomes, and raises the question whether the action of ubiquitinating/de-ubiquitinating attributes to sexual differentiation of the brain. Supported by MH59268, NS43196, HD43942.

**Down syndrome cell adhesion molecule (DSCAM): fetal heart expression is myocardial and mouse models provide clues to its role in cardiac development.** *G.M. Barlow<sup>1</sup>, K. Amano<sup>2</sup>, K. Yamakawa<sup>2</sup>, G. Lyons<sup>3</sup>, J.R.*

*Korenberg<sup>1</sup>*. 1) Med Genet, Cedars-Sinai Medical Center, UCLA, Los Angeles, CA; 2) Riken Brain Science Institute, Japan; 3) Dept. of Anatomy, Univ. of Wisconsin Medical School, Madison, WI.

Down syndrome (DS) is a major cause of congenital heart disease (CHD), particularly with defects of the endocardial cushions and AVSD (atrioventricular septal defect). We previously identified DSCAM as a candidate gene for DS-CHD, and identified a chromosome 11q gene homolog, DSCAML1 as a candidate gene contributing to the AVSD associated with Jacobsen syndrome caused by deletion of 11q. To examine the expression in the developing mouse heart, we found that both DSCAMs were expressed in the myocardium of the atria and ventricles from at least E10, but were not expressed in the endocardium or epicardium. Further, both DSCAM and DSCAML1 are excluded from the endocardial cushions prior to and during their fusion, and continue to be excluded from the cushion region until at least E16. We hypothesize that DSCAMs are involved in myocardial and cushion cell differentiation, and that its myocardial overexpression leads to decreased EC cell proliferation, premature differentiation, and defective cushion formation and contributes to cushion defects in both DS and del 11q.

To investigate the effects of altered DSCAM expression on heart development, we generated a putative dominant negative transgenic mouse model expressing a truncated DSCAM cDNA controlled by a 1.8 kb human DSCAM promoter region (Barlow et al., 2003) and a knockout for DSCAM. Previous analyses of our lacZ reporter models showed that this promoter drives expression in the developing brain and limb buds, but not in the developing heart. Analysis of hearts from the putative dominant negative and K/O mice revealed that they do not develop structural heart defects. Taken together, these results suggest that the elements required to drive DSCAM expression in the fetal heart lie outside of the 1.8 kb promoter region, and unusually, that the effects of DSCAM over expression may have more serious consequences, at least for cardiac development, than the effects of its deletion.

Program Nr: 906 from 2003 ASHG Annual Meeting

**Glomulin is Predominantly Expressed in Vascular Smooth Muscle Cells in the Embryonic and Adult Mouse.**

*B.A.S. McIntyre, P. Brouillard, V. Aerts, I. Gutierrez-Roelens, M. Vikkula.* Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology & Université catholique de Louvain, Brussels, Belgium.

Mutations in the glomulin gene result in dominantly inherited vascular lesions of the skin known as glomuvenous malformations (GVMs)(MIM#138000) (Brouillard et al., Am J Hum Genet 2002). These lesions are histologically distinguished by their distended vein-like channels containing characteristic "glomus cells", which appear to be incompletely or improperly differentiated vascular smooth muscle cells. The function of glomulin is currently unknown. We studied glomulin expression during murine development (E9.5 days post coitum until adulthood) by non-radioactive in situ hybridization. Glomulin was first detected at E10.5 dpc in cardiac outflow tracts. Later, it showed strong expression in vascular smooth muscle cells as well as a limited expression in the perichondrium. At E11.5-14.5 dpc glomulin RNA was most abundant in the walls of the large vessels. At E16.5 dpc expression was also detectable in smaller arteries and veins. The high expression of glomulin in murine vasculature suggests an important role for glomulin in blood vessel development and/or maintenance, which is supported by the vascular phenotype seen in GVM patients with mutations in this gene. (vikkula@bchm.ucl.ac.be) (<http://www.icp.ucl.ac.be/vikkula>).

**Primary cilia in polycystin-2 knockout mice.** *J. Horst*<sup>1</sup>, *P. Pennekamp*<sup>1</sup>, *G. Lewandowski*<sup>2</sup>, *C. Rasch*<sup>3</sup>, *R. Reichelt*<sup>3</sup>, *B. Dworniczak*<sup>1</sup>. 1) Inst Human Genetics, WWU Muenster, Muenster, Germany; 2) HistoServe, Muenster, Germany; 3) Institut fuer Medizinische Physik und Biophysik, Universitaetsklinikum Muenster, Germany.

Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disorder affecting 1 in 800 individuals. It is characterized by the formation of large, fluid-filled cysts in the kidney, the liver and other organs. Two genes have been identified which are mutated in 99% of ADPKD cases: PKD1, encoding polycystin-1 and PKD2, encoding polycystin-2. It has been shown that polycystins influence signalling pathways including the Wnt, G-protein and JAK/STAT pathways and that they are involved in Ca<sup>2+</sup> signalling. Both polycystins are imbedded in the kidney primary cilia membrane and recent data suggest that primary cilia function as cell antennae relaying environmental clues as calcium signals to the cell body. Primary cilia are found on a wide variety of cells in the body. The best known are cilia on the node a cellular structure that is involved in the development of normal left-right asymmetry in vertebrates. We have generated a *Pkd2* knockout mouse. Loss of *Pkd2* cause recessive embryonic lethality. *Pkd2*<sup>-/-</sup>*LacZ*<sup>+/+</sup>-embryos develop whole body edema and cysts in the kidneys, pancreas and liver. Mutant hearts exhibit structural deformations with abnormalities in trabecularization and cardiac septation. *Pkd2* knockout mouse embryos display L-R axis defects: embryonic turning, heart looping and placement of abdominal organs are randomized and the lung shows right pulmonary isomerism. *Lefty1*, *Lefty2* and *nodal* are not expressed in the lateral plate mesoderm and *Pitx2* is absent from heart and lung primordia and bilaterally expressed. We suggest that in mouse the ion channel polycystin-2 acts upstream of the nodal cascade in L-R axis determination. We hypothesize that loss of polycystin-2 affects the function of the monocilia on ventral cells of the node which are required upstream of the nodal cascade. Currently we are analysing structure and function of these monocilia and data concerning these experiments will be presented.

**A model for genetic interaction- Severe abnormalities in double heterozygotes for Zic3 and Nodal genes.** *K.G Harutyunyan, S.M. Ware, J.W. Belmont.* Molec & Human Genetics, Baylor College Medicine, Houston, TX.

Mutations in the *Zic3* gene, a member of the opa/Gli transcription factor superfamily, causes a rare developmental disease, X-linked heterotaxy (HTX1). *Zic3* null mice demonstrate abnormal left-right patterning, neural tube defects, and congenital heart defects. *Zic3* null mice therefore recapitulate many of the defects seen in patients with X-linked heterotaxy and are thus an important model for studying the mechanistic basis of this disease. Nodal, a TGF-beta family related growth factor, is a critical component in the determination of left-right asymmetry. Examination of Nodal gene expression in *Zic3* null mice demonstrated a failure to maintain Nodal expression as well as randomized expression of Nodal at the node and left lateral plate mesoderm. To determine whether *Zic3* might genetically interact with the Nodal pathway, we have intercrossed *Zic3*<sup>+/-</sup> and *Nodal*<sup>+/-</sup> mice. *Zic3*<sup>+/-</sup>/*Nodal*<sup>+/-</sup> compound heterozygote mice are born in statistically significantly reduced numbers compared to either single heterozygote; no *Zic3*<sup>null</sup>/*Nodal*<sup>+/-</sup> mice are born, indicating embryonic lethality in these mutants. However, only 50% of *Zic3* null die in utero and approximately 30% are lost in perinatal period. Nodal haploinsufficiency in *Zic3* deficient mice may allow us to identify a new functional role of these genes. Analyses of embryos from *Zic3*<sup>+/-</sup> and *Nodal*<sup>+/-</sup> crosses did not show any discrete time point of lethality. This result suggests that embryonic losses may occur over an extended time. Embryonic defects in *Zic3*<sup>+/-</sup>/*Nodal*<sup>+/-</sup> compound heterozygotes are phenotypically similar to those found in *Zic3* null embryos and include caudal truncation, neural tube defects, failure to complete heart looping and dextrocardia. The defects in *Zic3*<sup>null</sup>/*Nodal*<sup>+/-</sup> embryos are generally more severe than those found in *Zic3* null embryos. All these data demonstrate strong evidence of genetic interaction between these two genes. To elucidate the specific stage and site of this genetic interaction, we are undertaking an investigation of the effect of *Zic3* deficiency on Nodal expression in vivo using Nodal promoter reporter transgenes.

**Expression of Optineurin (OPTN), an Adult Onset Primary Open Angle Glaucoma (POAG) Gene, During Early Stages of Mouse Eye Development.** *I. Stoilov, T. Rezaie, M. Sarfarazi.* Molecular Ophthalmic Genetics Laboratory, Surgical Research Center, Department of Surgery, University of Connecticut Health Center, Farmington, CT.

Primary open-angle glaucoma (POAG) affects 33 million individuals worldwide and is one of the leading causes of blindness. Recently, we identified OPTN as the defective gene for locus GLC1E on chromosome 10p15-p14. Optineurin interacts with Huntingtin, Ras-associated protein RAB8, and Transcription Factor IIIA and has been implicated in the Tumor Necrosis Factor- signaling pathway. After cloning the mouse Optn gene our objective was to investigate expression of this gene during early stages of mouse development by whole mount In Situ hybridization. Mouse embryos from FVB/NCrlBR strain were fixed with 4% paraformaldehyde and dehydrated through methanol series. An Optn cDNA fragment was amplified by PCR and subcloned into transcription vector pCRII-TOPO (Invitrogen). Antisense and sense RNA probes labeled with digoxigenin-UPT were generated by in vitro transcription of linearized plasmids with SP6 and T7 RNA polymerase. The digoxigenin-labeled RNA was localized using a Fab fragments from an anti-digoxigenin antibody conjugated with alkaline phosphatase. Colorimetric detection was performed with NBT/BCIP substrate solution. We studied FVB mouse embryos staged at 9.0, 9.5, 10.5 and 12.5 dpc (days post conception). A distinct Optn expression domain was observed in the developing eye of 9.5, 10.5 and 12.5 dpc embryos. The Optn expression domain was identical to areas of pigmentation observed in the developing eyes of non-albino strains (C57BL/6). Inspection of sections from cephalic region of the whole mounts revealed that during the period of 9.5-12.5 dpc, the expression of Optn is restricted to outer layers of the optic cup (future pigment layer of retina). In summary, our study indicates that: 1)-Optn expression is triggered early during the eye development; 2)-Optn expression is localized in tissues corresponding to future pigment layers of the retina and; 3)-eye is a major site of Optn expression in the developing embryo. Supported by AHAF National Glaucoma Research grant and NIH (EY-09947).

**Breakpoint mapping of a *de novo* 15p;16p translocation reveals a candidate gene for autism.** C.L. Martin<sup>1</sup>, Y. Ilkin<sup>1</sup>, C. Powell<sup>2</sup>, K. Rao<sup>2</sup>, A. Whichello<sup>3</sup>, E. Cook<sup>1,4</sup>. 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Division Genetics and Metabolism, Univ North Carolina, Chapel Hill, NC; 3) Duke University School of Nursing, Durham, NC; 4) Dept Psychiatry, Univ Chicago, Chicago, IL.

A *de novo* translocation between the short arms of chromosome 15 and 16 was identified by G-banding analysis in a female with autism, epilepsy, delayed walking with mild residual ataxia, a history of behavioral regression, fluctuating liver function tests and mild cerebellar atrophy (sample from the AGRE collection). Fluorescence *In Situ* Hybridization (FISH) analysis using a set of genomic clones spaced across the most distal 10 Mb of 16p was used to localize the translocation breakpoint between 5 and 7 Mb from the telomere. Additional targeted FISH mapping was carried out in this region and identified a cryptic deletion of approximately 160 kb at the site of the translocation breakpoint. The deletion is at the boundary of the first exon and the first intron of the 1.7 Mb ataxin-2 binding protein-1 (*A2BP1*) gene which binds to ataxin-2, implicated in Spinocerebellar Ataxia type 2 (SCA2). As a result of the translocation, exon 1 of the *A2BP1* gene is moved to 15p while the rest of the gene remains on 16p. The *A2BP1* gene encodes a 377 amino acid protein and is highly conserved in *C. elegans*, *D. melanogaster*, mouse and human. *A2BP1* has been previously characterized by another group and shown to code for an RNA-binding protein which is expressed in muscle and brain. Specifically in the brain, *A2BP1* is found in the cytoplasm of Purkinje cells and dentate neurons in a punctate pattern. In the mouse, *A2bp1* is expressed in the neurons of the cerebral cortex, cerebellum, hippocampus and brain stem. This expression pattern is similar to the pathological pattern observed in brain tissue of autism patients. Therefore, it is likely that the rearrangement involving *A2BP1* in this child is responsible for her phenotype. In addition, given the function and expression pattern of *A2BP1*, it could be a candidate gene for other cases of autism.

Program Nr: 911 from 2003 ASHG Annual Meeting

**Tissue and developmental specificity of chromosomal homolog positioning in the interphase nucleus.** *S.R. Rittling, M. Mamcarz, J.A. Tischfield.* Genetics, Rutgers University, Piscataway, NJ.

Chromosomes occupy distinct, non-overlapping territories in the interphase nucleus. Studies on mitotic recombination between homologous regions on the two chromosomes 8 in mouse tissues reveal that the frequency of mitotic recombination is tissue specific. Since recombination in this model requires physical interaction between the two chromosomes, we reasoned that differences in the interhomolog spacing in different tissues could partially account for the differences in the frequency of mitotic recombination. Chromosome 8 territories were labeled using chromosome paints in interphase nuclei in freshly explanted cells from spleen, ear and kidney as well as in cultured ES cells. The closest physical distance between the two territories was measured in three-dimensions, as well as the distance of each territory from the nuclear periphery. Initial results indicate that while the closest spacing of the two territories does not correlate with mitotic recombination frequency, there is tissue specificity in the arrangement of chromosome territories in the different tissues.

**Identification of KIAA1034 as the cleft palate gene on 2q32-q33.** *D.R. FitzPatrick<sup>1</sup>, J. Leek<sup>2</sup>, L. McLaren<sup>1</sup>, I. Carr<sup>2</sup>, N. McGill<sup>1</sup>, J. Fantes<sup>1</sup>, A. Markham<sup>2</sup>, P. Wightman<sup>1</sup>, K. Williamson<sup>1</sup>, P. Gautier<sup>1</sup>, D.T. Bonthron<sup>2</sup>.* 1) MRC Human Genetics Unit, Edinburgh, United Kingdom; 2) Molecular Medicine Unit, Leeds, United Kingdom.

Cytogenetic evidence, in the form of deletions and balanced translocations, points to the existence of a locus on 2q32-q33, for which haploinsufficiency results in isolated cleft palate (CPO). Here we show by high resolution FISH mapping of two de novo CPO-associated translocations involving 2q32-q33 that one breakpoint interrupts the transcription unit of the gene encoding the DNA-binding protein KIAA1034. The breakpoint in the other translocation is located 130 kb 3 prime to the KIAA1034 polyadenylation signal, within a conserved region of non-coding DNA. The KIAA1034 gene is transcribed in a telomeric to centromeric direction and lies in a gene-poor region of 2q32-q33; the nearest confirmed gene is 1.26 Mb centromeric to the KIAA1034 polyadenylation signal. KIAA1034-encoding transcripts are assembled from 11 exons that span 191 kb of genomic DNA. They encode a protein of 733 amino-acids that has two CUT domains and a homeodomain and shows a remarkable degree of evolutionary conservation, with only 3 amino acid substitutions between mouse and human. This protein belongs to the same family as SATB1, a nuclear matrix-attachment region binding protein implicated in transcriptional control and control of chromatin remodelling. There are also sequence similarities to the *Drosophila* protein DVE. Whole mount in situ hybridisation to mouse embryos shows site- and stage-specific expression of KIAA1034 in the developing palate. Despite the strong evidence supporting an important role for KIAA1034 in palate development, mutation analysis of 70 unrelated patients with CPO did not reveal any coding region variants.

**Developmental origin of cultured chorionic villi: implications for cytogenetics.** *P.J. Yong<sup>1</sup>, D.E. McFadden<sup>2</sup>, C.D. MacCalman<sup>3</sup>, W.P. Robinson<sup>4</sup>.* 1) MD/PhD program; 2) Departments of Pathology; 3) Obstetrics; 4) Medical Genetics, UBC, Vancouver, Canada.

Cytogenetic analysis of the placenta can be indicated for both pregnancy management (via CVS) and investigation of pregnancy loss. Placental karyotyping is performed on cell cultures of chorionic villi, which are composed of an inner mesenchyme and an outer epithelium (trophoblast) derived from the inner cell mass (ICM) and trophoblast of the early embryo, respectively. Cells from these cultures are generally thought to be from the ICM-derived mesenchyme. However, previous studies using the epithelial marker cytokeratin-18 (CK18) have shown some CK18-positive cells in such cultures. Recently, a tissue histological study showed some CK18 expression in the mesenchyme, but identified a second epithelial marker, cytokeratin-7 (CK7), that was expressed only in the trophoblast.

We have assessed CK7 and CK18 protein expression in cultures of chorionic villi from CVS (n = 6) and from miscarriages (n = 5) using standard immunocytochemistry. Cultures showed few or no CK7-positive cells (0.4%0.7% [s.d.]) but variable proportions of CK18-positive cells (19%17%) (p=0.0025). There were no significant differences in the proportion of CK7- or CK18-positive cells based on karyotype or ascertainment (CVS vs miscarriage). Interestingly, a culture with maternal contamination (shown by PCR), which was excluded from the analysis, showed a high level (27%) of CK7-positive cells.

Hence, the vast majority of cells from chorionic villi cultures were true mesenchymal cells expressing neither the CK7 nor CK18 epithelial markers, while CK7 expressing cells (trophoblast) were very rare. This confirms that karyotypes from such cultures are representative of the ICM. The subpopulation expressing CK18 but not CK7 is presumably also ICM-derived, but may be a group of epithelial-like cells with a unique developmental history. They may be significant when only a few cells are karyotyped or when mosaicism is evident. Furthermore, CK7 expression may serve as a marker of maternal contamination likely originating from endometrial gland epithelium.

**Allelic variations at the haploid TBX1 locus do not influence the cardiac phenotype of 22q11 microdeletion.** *N. Philip<sup>1</sup>, L. Girardot<sup>1</sup>, B. Giusiano<sup>2</sup>, N. Levy<sup>1</sup>, M.A. Voelckel<sup>1</sup>.* 1) Dept de Genetique Medicale, Hosp d'Enfants de la Timone, Marseille Cedex 5, France; 2) Service d'information Mdicale, Hospd'Enfants de la Timone, Marseille.

Microdeletion at the 22q11 locus is characterised by a high clinical variability. Congenital heart defects are the most life-threatening manifestations of the syndrome and affect approximately 50% of patients. The causes of this phenotype variability remain unknown although several hypothesis may be raised, including a potential role modifier genes, a specific genetic background, environmental factors and epigenetic modifications. It has been suggested that allelic variations at the haploid locus could modify the phenotypic expression. Regarding this hypothesis, TBX1 was thought to be a major candidate to the cardiac phenotype or its severity in patients carrying the 22q11 microdeletion. To further explore this hypothesis a mutational screening was performed in TBX1 coding exons and flanking intronic sequences, in a series of 39 patients with a 22q11 microdeletion, proved by FISH analysis. Two groups were delineated, according to the cardiac status. 23 had a congenital heart defect (5 interrupted aortic arch; 4 truncus arteriosus; 7 TOF, 7 VSD). 16 had a normal cardiac examination. TBX1 Gene analysis was performed by PCR-SSCP method followed by sequencing analysis of the PCR product. Our results did not show any significant mutation in the TBX1 gene, in our population of deleted patients. We identified 5 different nucleotide substitutions in 17 patients in the haploid TBX1 gene : 8 patients carried a single substitution, 8 patients carried 2 different changes on the non deleted chromosome and a single patient had 3. There were no differences in the distribution of these polymorphisms between the two groups of patients. Four of the sequence variations identified corresponded to polymorphisms previously reported by Gong et al (2002). Moreover, we carefully screened these nucleotides substitutions for putative donor splice sites and did not found any. These results are against a major role of TBX1 polymorphisms at the haploid locus, in the pathogenesis of cardiac defects in 22q11 microdeletion.

**Mutational analysis of the *TP63* gene in Mexican patients with syndromic and isolated ectrodactyly.** *G. Pozo-Molina*<sup>1</sup>, *V. Berdon-Zapata*<sup>1</sup>, *M. Granillo-Alvarez*<sup>1</sup>, *M. Valdes-Flores*<sup>2</sup>, *J.E. Garcia-Ortiz*<sup>3</sup>, *S. Canun*<sup>4</sup>, *N. Perez*<sup>4</sup>, *S. Kofman-Alfaro*<sup>1</sup>, *J.C. Zenteno*<sup>1</sup>. 1) Department of Genetics, General Hospital of Mexico-Faculty of Medicine UNAM, Mexico City, Mexico; 2) Department of Genetics, Centro Nacional de Rehabilitacion, Mexico City, Mexico; 3) Department of Molecular Immunobiology, CIB, UadeC, Torreon, Mexico; 4) Department of Genetics, Hospital "Manuel Gea Gonzalez", Mexico City, Mexico.

Ectrodactyly is a congenital limb malformation that involves a central reduction defect of the hands and/or feet which is frequently associated with other phenotypic abnormalities. The condition is genetically heterogeneous and recently it has been demonstrated that mutations in the *TP63* gene, a homologue of the archetypal tumor suppressor gene *TP53*, are the cause of four autosomal dominant syndromes which feature ectrodactyly: EEC (ectrodactyly, ectodermal dysplasia, and facial clefting), isolated SHFM (split-hand/split-foot malformation), LMS (limb-mammary syndrome), and ADULT (acro-dermato-ungual-lacrimal-tooth syndrome). In this study we report the results of genetic analysis of the *TP63* gene in 17 Mexican individuals with syndromic and isolated ectrodactyly. Four patients with syndromic ectrodactyly had *TP63* heterozygous point mutations: in a patient with EEC syndrome, we found a CGC to CAC mutation in exon 7 which predicted an arginine to histidine change at position 279 of TP63 (R279H), located in the DNA binding domain of the protein; in other two patients (a mother with an EE phenotype and her daughter with EEC syndrome), a change from CGG to TGG at codon 204 in exon 5 predicted a shift from arginine to tryptophan (R204W), also in the DNA binding domain; a third mutation was identified in another patient with an EE phenotype and was identical to that described in the first subject (R279H). Finally, a missense mutation located at exon 3 was found in a subject with isolated ectrodactyly; this mutation predicts a R97C shift in the transactivation (TA) domain of TP63 and is the first naturally occurring mutation described in the TA domain. We discuss on the phenotype-genotype correlation observed in this and previous studies.

**The human hairless protein interacts with the 20S proteasome.** A. Hillmer<sup>1</sup>, U. Heyn<sup>1</sup>, G.M. Beaudoin<sup>2</sup>, R.C. Betz<sup>3</sup>, C.C. Thompson<sup>2</sup>, S. Cichon<sup>3</sup>, M.M. Noethen<sup>3</sup>. 1) Inst. of Human Genetics, Univ. of Bonn, Bonn, Germany; 2) Dept. of Neuroscience, Johns Hopkins Univ. School of Medicine/Kennedy Krieger Institute, Baltimore, MD, USA; 3) Dept. of Medical Genetics, Univ. of Antwerp, Antwerp, Belgium.

Mutations in the human hairless gene are responsible for complete, congenital absence of hair in families with autosomal recessive universal congenital alopecia (MIM #203655) and autosomal recessive papular atrichia (MIM #209500). These findings clearly show that the hairless protein (HR) is essential and specific for the maintenance of human hair. Little is known about the functional and structural properties of HR. The rat hairless protein (Hr) functions as a transcriptional corepressor for thyroid hormone receptors. To shed light on the function of HR, we identified HR-interacting proteins using yeast two-hybrid screens of a keratinocyte cDNA library. We identified the 20S proteasome subunit -7 (Pro-7) as a potential interaction partner of HR. The existence of this interaction *in vivo* was confirmed by co-immunoprecipitation of HR and Pro-7 expressed in COS-1 extracts, and GST pulldown experiments indicate that the interaction is direct. The domain of Hr which is responsible for the interaction with Pro-7 has been mapped in yeast two-hybrid and co-immunoprecipitation assays, indicating that amino acids 180-200 of Hr (corresponding to aa 154-174 of the human protein HR) are responsible for the interaction. To establish the functional significance of HR:Pro-7 interaction, we determined whether Hr is degraded by the proteasome. To reduce protein degradation by the proteasome, Hr expressing COS-1 cells were treated with a proteasome inhibitor. We found that increasing time of incubation with the proteasome inhibitor results in an increase of Hr, indicating that Hr is more stable when proteasome activity is decreased, suggesting that Hr is normally degraded by the proteasome. Although the biological relevance of this Hr degradation is unclear, it is possible that cyclic degradation of Hr is required for maintaining the cyclic transformations of the hair follicle. Future studies are needed to clarify this issue.

**Analysis of gene expression patterns in myogenic cells from healthy subjects and Duchenne Muscular Dystrophy patients.** *J.T. den Dunnen<sup>1</sup>, E. Sterrenburg<sup>1</sup>, R. Turk<sup>1</sup>, J.M. Boer<sup>1</sup>, F. Baas<sup>2</sup>, J.C.T. van Deutekom<sup>1</sup>, G.J.B. van Ommen<sup>1</sup>, P.A.C. 't Hoen<sup>1</sup>.* 1) Human and Clinical Genetics, Leiden University Medical Center, Leiden, Nederland; 2) Department of Neurology, Academic Medical Center, Amsterdam, Nederland.

To study myogenesis in general and disturbances in this process caused by defects in the genes involved in neuromuscular disorders, we are determining genome-wide gene expression levels using array technology. Cultured human satellite cells of healthy controls and patients are induced to form myofibers through serum depletion. RNA is isolated at different time-points after induction. Gene expression levels are measured using a 5,000-feature muscle-expressed and a 20,000-feature general micro-array. In cells from healthy individuals, as expected, serum depletion downregulates genes involved in proliferation and DNA replication, while genes related to energy metabolism and cell cycle arrest are upregulated. Genes involved in muscle development show a gradual upregulation, with a distinct set of genes showing highest expression around the time the cells start to fuse. Clusters of potentially co-regulated transcripts that, over time, behave in the same way contain EST sequences encoding unknown functions, their clustering providing clear hints towards their potential role. To verify whether processes known to be disturbed in neuromuscular disorders could be visualised, we zoomed in on the expression of genes that encode parts of the Dystrophin-associated Glycoprotein Complex (DGC). Our results show a downregulation of several of these genes in cells from Duchenne muscular dystrophy (DMD) patients. Current analysis focuses on the identification of other disturbances and whether these can be used for diagnostic purposes. In addition we are analysing gene expression profiles of non-myogenic cells, with or without myoD-induced forced myogenesis, to determine whether these can be used to study the same processes.

**Folic acid suppression of nicotine-induced teratogenesis in zebrafish embryos .** *S. Beiraghi, M.A. Pickart, E. Beckman, D. Shiroma, S.C. Ekker.* The Arnold and Mabel Beckman Center for Transposon Research, University of Minnesota, Minneapolis, MN.

Children of mothers that smoke have an increased risk for developmental abnormalities such as low birth weight as well as specific craniofacial anomalies including cleft lip and palate (Romotti et al 1998). Previous reports describe the teratogenic potential of nicotine in developing mouse embryos. We have extended this observation to zebrafish embryos. Independent animal studies and human epidemiologic intervention trials have demonstrated the capacity of folic acid to decrease the incidence of neural tube defects and orofacial clefts. **Objective:** The objective of this work was to identify the minimal concentration of nicotine necessary to alter the morphology of developing zebrafish embryos and to characterize the phenotypes observed. Subsequent studies examined the hypothesis that folic acid can suppress the teratogenic effects of nicotine. **Methods:** Within 1.5 hours post-fertilization, zebrafish embryos were bathed in 1.25 mM or 1.5 mM L-nicotine in the absence or presence of folic acid (concentrations between 0.05 mM and 1.0 mM) for 24 hours. Control (untreated) embryos were bathed in egg water. After 24 hours of treatment, embryos were transferred to new dishes and bathed in egg water. Embryo morphology was assessed at 48 hours and 5 days. **Results:** Embryos exposed to 1.25 mM and 1.5 mM nicotine displayed a spectrum of developmental malformations in more than half of the 48 hour and 5 day old fish. Treatment of folic acid with nicotine displayed a dose dependent suppression of the teratogenic effects of nicotine, however, only 1.0 mM folic acid was effective at suppressing the effects of both 1.25 and 1.5 mM nicotine. **Conclusion:** Our preliminary data supports the view that nicotine is a potent teratogen, particularly affecting early vertebrate embryogenesis. Furthermore, it suggests that folic acid may be an effective agent to counteract the negative effects of nicotine during vertebrate.

**Dax1/Nr0b1 and network partners are expressed in embryonic development prior to previously established function in steroidogenic axis ontogenesis.** *R. Clipsham*<sup>1</sup>, *K. Niakan*<sup>2</sup>, *E.R.B. McCabe*<sup>1,2,3</sup>. 1) Molecular Biology Institute at UCLA, LA, CA; 2) Dept of Human Genetics, David Geffen Sch of Med at UCLA, LA, CA; 3) Dept of Pediatrics, David Geffen Sch of Med at UCLA and Mattel Children's Hosp, LA, CA.

Mutations in *NROB1*, the gene encoding DAX1, are responsible for X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. Duplication of the *NROB1* region results in 46,XY sex-reversal. Targeted deletion of *Nr0b1* is thought to be embryonic lethal and transgenic over-expression on a *Mus domesticus poschiavinus* (weak Sry) background results in XY sex-reversal. We hypothesized that DAX1/Dax1 functions in embryogenesis prior to steroidogenic axis ontogenesis. The purpose of this investigation was to determine expression of *Nr0b1* and its network partners in both cultured embryonic stem (ES) cells and preimplantation embryos. We analyzed expression of *Nr0b1* and its key associated network partners, steroidogenic factor 1 (*Nr5a1/Sf1*) and Wilms tumor 1 (*Wt1*), in cultured ES cells in the totipotent and differentiated states by northern blot hybridization and RT-PCR, as well as *in vivo* by immunohistochemistry (IHC) of preimplantation embryos. *Nr0b1*, *Sf1* and *Wt1* were highly expressed in totipotent ES cells, with reduced expression following induction toward individual germ layer fates. Expression of Dax1 and network partners was observed by whole-mount IHC analysis of preimplantation embryos flushed from the uterus at 4.5 days post coitum. These findings are consistent with the existence of a potentially functional network of transcription factors, including Dax1, very early in embryonic development. These results validate ES cells as a developmentally dynamic model for investigations into potential roles for this regulatory network early in embryogenesis preceding organogenesis. We speculate that DAX1/Dax1 and other members of this transcriptional network have novel and possibly pleiotropic functions in early embryonic development.

**Analysis of hypothalamic-pituitary-adrenal axis development in zebrafish.** Y. Zhao<sup>1</sup>, N. Liu<sup>2</sup>, J.K. Phelan<sup>3</sup>, Z. Yang<sup>4</sup>, S. Lin<sup>4</sup>, E.R.B. McCabe<sup>1,3,5</sup>. 1) Dept Hum Gen, David Geffen Sch Med at UCLA, LA, CA; 2) Dept Med, Cedars-Sinai Res Inst, LA, CA; 3) Dept Peds, David Geffen Sch Med at UCLA, LA, CA; 4) Dept Molec, Cell and Dev Bio, UCLA, LA, CA; 5) Mattel Children's Hospital at UCLA, LA, CA.

Molecular development of the hypothalamic-pituitary-adrenal axis (HPA) is only partially understood. The zebrafish is model for vertebrate development. Proopiomelanocortin (*pomc*), a pituitary prohormone, and *ff1b* (*nr5a4*), a homologue of the steroidogenic factor-1 (*SF1*, *NR5A1*), are essential for HPA development. Our purpose was to compare expression patterns of *ff1b* and *pomc* in zebrafish early development. *In situ* hybridization was performed on embryos at ten developmental stages with *ff1b* riboprobes. Expression of *ff1b* was first detected in two narrow strips of cells extending from rostral basal forebrain midline at 18-somites. Two separate groups of *ff1b* expressing cells were first observed in the mid-abdominal region at 22 hours post fertilization (hpf), and the two sites of expression coalesced by 28 hpf. To compare temporal and spatial expression patterns of *ff1b* and *pomc*, two-color *in situ* hybridization was performed at 24 and 36 hpf embryos with riboprobes labeled with fluorescein (*ff1b*) and digoxigenin (*pomc*). In rostral basal forebrain, *pomc* expression was caudal to *ff1b* within the same area, but without significant overlap, and the second *ff1b* expression domain was confirmed in the mid-abdominal region. Based on *ff1b* and *pomc* expression in fish, and extrapolating from their known expression patterns in mammals, these transcripts represent markers for HPA during development: *ff1b* for hypothalamus and adrenal cortex, and *pomc* for pituitary. To evaluate known candidate genes, and to identify additional genes involved in HPA development using forward genetic screens, we are in the process of generating transgenic zebrafish that express fluorescent protein markers under control of *ff1b* and *pomc* promoters. One line of fish (*ff1b-1*) expresses green fluorescent protein (GFP) in adrenal cortex, but interestingly not hypothalamus. Identification of genes involved in HPA development in zebrafish will inform our understanding of pathogenesis of developmental disorders involving human HPA.

**Regulation of progenitor cell fusion by ABCB5 P-glycoprotein, a novel human ATP-binding cassette transporter.**

*N.Y. Frank*<sup>1</sup>, *S. Pendse*<sup>2,3</sup>, *P.H. Lapchak*<sup>3</sup>, *D. Shlain*<sup>3</sup>, *C. Doeing*<sup>3</sup>, *M.H. Sayegh*<sup>2,3</sup>, *M.H. Frank*<sup>2,3</sup>. 1) Partners Center for Human Genetics, Harvard Medical School, Boston, MA; 2) Laboratory of Immunogenetics and Transplantation, Dept. of Medicine, Brigham & Women's Hospital, Boston, MA; 3) Division of Nephrology, Children's Hospital, Boston, MA.

Cell fusion involving progenitor cells is a newly recognized phenomenon thought to contribute to tissue differentiation and repair. The molecular mechanisms governing cell fusion are unknown. P-glycoprotein (P-gp) and related ATP-binding cassette (ABC) transporters are expressed by progenitor cells, but their physiological role in these cell types has not been defined. Here, we have cloned and characterized a novel, third member of the human P-gp family encoded on chromosome 7p21-15.3, designated ABCB5 P-gp, which marks CD133-expressing progenitor cells among human epidermal melanocytes and determines the capacity of this cell subset to undergo cell fusion. Vector-mediated ABCB5 gene transfection and expression in MCF-7 breast carcinoma cells, which at baseline do not express the molecule as determined by the polymerase chain reaction and flow cytometry, demonstrated that ABCB5 P-gp mediates rhodamine-123 transport, a hallmark P-gp function. Intriguingly, magnetic microbead-purified ABCB5 P-gp-positive cells contained from one to four nuclei by fluorescent microscopy and 2n, 4n, 6n or 8n amounts of DNA as determined by propidium iodide staining and flow cytometry. DiI/DiO membrane dye labeling studies revealed that polyploid ABCB5-positive cells are generated by cell fusion and that specific anti-ABCB5 P-gp monoclonal antibody (mAb)-mediated blockade, but not isotype control Ab or anti-ABCB1 (MDR1) mAb treatment, significantly enhanced this process (p0.05). Remarkably, multinucleated cell hybrids gave rise to mononucleated progeny during continued culture, demonstrating that fusion contributed to culture growth and differentiation. Thus, our results define a novel molecular mechanism for cell fusion involving progenitor cells and show that fusion and resultant growth and differentiation are not merely spontaneous events, but phenomena specifically regulated by ABCB5 P-gp function.

**EDA-A1 transgene-induced guard hair formation and sebaceous gland hyperplasia.** C. Cui<sup>1</sup>, T. Hashimoto<sup>1</sup>, M. Durmowicz<sup>1</sup>, C. Ottolenghi<sup>1</sup>, B. Griggs<sup>2</sup>, A.K. Srivastava<sup>2</sup>, D. Schlessinger<sup>1</sup>. 1) Laboratory Genetics, NIA/NIH, Baltimore, MD; 2) J.C. Self Research Institute of Human Genetics, Greenwood Genetic Center, Greenwood, SC.

Mutations in the X-linked EDA gene cause Anhidrotic Ectodermal Dysplasia (EDA), with lesions in skin appendage formation; and mutations in the equivalent mouse gene, Ta, give a similar Tabby phenotype. EDA splice isoforms EDA-A1 and EDA-A2 belong to the TNF ligand family and function by activating NF- $\kappa$ B- and JNK- based transcription, consistent with microarray analyses (HMG 2001). To analyze their action further, we expressed the isoforms conditionally as tetracycline-regulated transgenes in Tabby and wild-type mice. High levels of expression of an mEDA-A1 transgene during embryogenesis had both determinative and trophic effects. The determination of sweat glands and one type of hair follicle (guard hair) was fully restored, in agreement with reported comparable effects of free circulating chimeric mEDA-A1 ligand (Nat Med 2003). In contrast, zigzag hair formation was not restored in Tabby mice and was sharply inhibited in wild-type mice. As a major trophic effect, mEDA-A1 has now been found to provoke hyperplasia of sebaceous glands in Tabby mice, with correspondingly higher levels of sebum formation. The trophic effect, like the initiating effects, required the presence of the isoform during embryonic life; but in contrast to the determinative effects, it was reversed when the gene was repressed in adult animals. In addition, none of the effects seen with EDA-A1 were given by comparable expression of the mEDA-A2 isoform. The results thus reveal trophic as well as initiating action of the EDA gene, and suggest a possible balance of isoform interactions in skin appendage formation and maintenance.

**Split Hand Foot Malformation is associated with a reduced level of Dactylin gene expression.** *D. Basel*<sup>1</sup>, *A. DePaepe*<sup>2</sup>, *M.W. Kilpatrick*<sup>1</sup>, *P. Tsipouras*<sup>1</sup>. 1) Dept Pediatrics, UConn Health Ctr, Farmington, CT; 2) University of Gent, Belgium.

Split Hand Foot Malformation (SHFM) is a developmental defect of the distal extremity presenting with median clefting of the hands and feet, syndactyly, and occasionally polydactyly. If severe, all four extremities are involved but usually only the hands are affected. SHFM is genetically heterogeneous and at least four different loci have been identified: SHFM1 (7q21.2-22.1), SHFM2 (Xq26), SHFM3 (10q24-25), and SHFM4 (3q27). SHFM3 has been localized to the 10q24 region but comprehensive mutation screening of several candidate genes within the critical region failed to identify any causative coding sequence alterations. Dactylaplasia (Dac), a semi-dominant trait in the mouse, which phenotypically resembles SHFM, has been described in a region syntenic to 10q24. This phenotype is associated with reduced levels of dactylin, an F-box/WD40 repeat containing protein which acts as an adaptor for proteins destined for ubiquitination. Transcript levels were determined for Dactylin and seven genes in its proximity. A significant difference between the expression profiles of five affected individuals and control samples was detected for two gene transcripts, Dactylin ( $p=0.0001$ ) and LBX1 ( $p=0.0002$ ). However, no significant difference was detected for these transcripts in the remaining six samples from affected individuals, Dactylin ( $p=0.442$ ) and LBX1 ( $p=0.340$ ). For six transcripts TLX1, BTRC, FGF8, NFKB2, CHUK and SUFU, no significant difference was observed between affected and control samples ( $p=0.473$ ,  $p=0.284$ ,  $p=0.437$ ,  $p=0.126$ ,  $p=0.157$  and  $p=0.152$  respectively). However, the transcript levels for both BTRC and FGF8 were low. The two-fold decrease detected in the level of Dactylin gene transcript from five unrelated individuals affected with SHFM as compared to unaffected controls supports a central role for dactylin in the pathogenesis of the disease and the possibility of a common pathogenesis for SHFM3 and Dac. Reduced levels of dactylin expression could result in insufficient removal of a postulated suppressor of cell proliferation leading to a failure of proliferative function in the Apical Ectodermal Ridge.

**Gene Expression Profiling of Human Pharyngeal Arches.** *J. Cai*<sup>1</sup>, *D. Ash*<sup>1</sup>, *L. Kotch*<sup>1</sup>, *E.W. Jabs*<sup>1</sup>, *T. Attié-Bitach*<sup>2</sup>, *J. Augé*<sup>2</sup>, *S. Audollent*<sup>2</sup>, *M. Vekemans*<sup>2</sup>, *Y. Korshunova*<sup>3</sup>, *R. Tidwell*<sup>3</sup>, *R. Veile*<sup>3</sup>, *D. Messina*<sup>3</sup>, *C. Helms*<sup>3</sup>, *M. Lovett*<sup>3</sup>. 1) Johns Hopkins Univ, Balto, MD; 2) Hospital Necker Enfants Malades, Paris, France; 3) Washington Univ, St Louis, MO.

The COGENE consortium is profiling gene expression to study craniofacial development. Twelve microSAGE libraries, averaging 50,000 tags per library, were constructed from microdissected structures of 4 to 8.5 wks of human development, including pharyngeal arch 1 (PA1) that forms the majority of the upper and lower jaw. Ninety-six of the 130 genes known to be expressed in mouse PA1 were identified, validating this SAGE dataset. Pairwise comparison between SAGE libraries revealed that 173 tags, corresponding to unique mRNAs, were upregulated, and 129 tags were downregulated ( $p < 0.05$ ) from 4 to 5 wks PA1, many of which are involved in transcriptional regulation. By comparing these SAGE libraries, genes that are expressed higher in pharyngeal arches than in any other structures (e.g. PA3 and 4, frontonasal prominence, anterior and posterior rhombomeres) were found. Many of these genes were previously unknown to be expressed in PA1. Whole mount *in situ* hybridization was performed on mouse embryos at corresponding stages to confirm the expression pattern observed in humans. For example, the *Cct3* gene, a molecular chaperone, was expressed highly in PA1. The *Set* gene, which plays a role in cell proliferation, was highly expressed both in PA1 precursor cells at GD8.5 and in PA1 at GD9.5, while the expression was more restricted to the maxillary portion of PA1 at GD10.5, making it a candidate gene for cleft lip/palate. Many of the expression changes were confirmed by Affymetrix microarrays and real-time RT-PCR. These data show conservation of expression patterns in human and mouse PA1 development. Many genes expressed in PA1 were also expressed in limb buds. Since craniofacial disorders are often associated with limb abnormalities, these findings suggest that similar signaling molecules are involved in both pharyngeal arch and limb development. This dataset should provide useful information in identifying pathways and genes that contribute to common pharyngeal arch defects including cleft lip and palate.

**Downstream effector candidates of HOX proteins in mammals.** *J.W. Innis*<sup>1, 2</sup>, *T.M. Williams*<sup>1</sup>, *R. Kuick*<sup>2</sup>, *D. Misek*<sup>2</sup>, *M.E. Williams*<sup>1</sup>, *L. Privette*<sup>1</sup>, *K. McDonagh*<sup>3</sup>, *S. Hanash*<sup>2</sup>. 1) Department of Human Genetics, University of Michigan; 2) Department of Pediatrics; 3) Department of Internal Medicine.

*Hox* genes encode transcription factors that regulate the morphogenesis of developing embryos. Knowledge of the genes and cis-acting sequences through which these highly conserved proteins regulate development is limited. Cell adhesion molecules and their receptors are known effectors of *Hox* expression, yet few other genes regulated by HOX proteins have been identified in mammals despite the broad developmental effects HOX proteins exert on cells. To identify genes whose expression is regulated by posterior HOX proteins, we expressed HOXA13 in a mouse mesenchymal cell line (NIH3T3-GP+E86) that lacks expression of any group 13 paralogue using a bicistronic HOXA13/EGFP retroviral vector. We applied EGFP-based fluorescence activated cell sorting to cells stably expressing HOXA13 and EGFP or EGFP alone and prepared RNA from the enriched populations. Utilizing Affymetrix Mouse Gene Arrays, we identified 50 genes that exhibited significantly ( $p < .01$ ) increased or decreased expression of at least two-fold in HOXA13-expressing cells. Among these, genes involved in BMP signaling, extracellular matrix and membrane metabolism, and numerous cell-surface molecules predominated. One zinc-finger transcription factor was upregulated 30-fold. Differences were confirmed for 10/11 selected candidates by semi-quantitative RT-PCR with independent RNA preparations, and RNA expression was demonstrated in the developing limb bud and mature cervix/vagina, two regions of known *Hoxa13* expression, for all 11 candidates. Additional whole mount *in situ* hybridization with *Hoxa13* mutants is being used to test whether *in vivo* expression of these candidate genes is altered in the mutant background. This work identifies specific genes involved in BMP signaling, extracellular matrix molecules and membrane/cell surface metabolism as strong candidates for regulation by posterior HOX proteins in morphogenesis.

**TGIF, a gene associated with holoprosencephaly, regulates neuronal gene expression.** *J.E. Ming<sup>1</sup>, A.C. James<sup>1</sup>, J.A. Golden<sup>2</sup>, J.L. Knepper<sup>1</sup>.* 1) Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia and the University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Department of Pathology, The Children's Hospital of Philadelphia, Philadelphia, PA.

Holoprosencephaly (HPE) is a developmental disorder of the forebrain resulting in incomplete separation of the cerebral hemispheres into distinct right and left halves. Mutations in *TGIF*, which encodes TG-interacting factor, have been detected in patients with HPE. TGIF is an atypical homeodomain protein. In vitro studies have demonstrated that TGIF acts as a transcriptional co-repressor in TGF-beta signaling. TGIF can also bind to a retinoid X receptor response element and compete for binding with RXR. However, the role that TGIF plays in central nervous system development and its relationship to the pathogenesis of HPE are unknown. As an initial step in assessing its function, we determined its expression pattern during nervous system development. We find that TGIF is expressed in discrete domains in the forebrain and spinal cord, with highest levels of expression in dorsal regions. Using electroporation in the chick embryo model, we ectopically expressed TGIF in the neural tube. This caused a decrease in the expression of the dorsally expressed transcription factors Pax6, Cath1, and Lhx2b. In contrast, the expression of other transcription factors, including those necessary for ventral fate such as Nkx2.2, were not affected. Thus, TGIF specifically represses the expression of genes expressed in specific dorsal-ventral domains of the neural tube. Studies examining the functional effect of the *TGIF* mutations identified in patients with HPE are ongoing. Our data suggest that TGIF plays an important role in regulating gene expression in developing neurons and that altering TGIF activity results in defective nervous system development.

**Late-Gestation-Lung 1 (LGL1) Levels are Diminished in Animal Models of Bronchopulmonary Dysplasia (BPD) and Normalized During Recovery in Air.** *K. Nadeau<sup>1,2,3</sup>, R. Jankov<sup>4,5,6</sup>, L. Oyewumi<sup>5,6</sup>, K.A. Tanswell<sup>4,5,6</sup>, N.B.*

*Swezey<sup>5,6,7</sup>, F. Kaplan<sup>1,2,3,8</sup>.* 1) Dept Human Genetics; 2) Children's Res Inst, Montreal, PQ, Canada; 3) Mtl. Child. Hosp. Res. Inst, Montreal, QC, Can; 4) Lung Bio and Med. Res. Council Group in Lung Dev; 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.

Malformation or destruction of alveoli characterize lung diseases of developmental origin (BPD). We cloned LGL1 (gene), a glucocorticoid induced and developmentally regulated gene in fetal and neonatal lung. The lg11 protein is a mesenchyme specific secreted glycoprotein. We showed that LGL1 regulates lung branching. However, maximal fetal LGL1 expression occurs in late gestation when branching of conducting airways is complete. Postnatally, lg11 (protein) is taken up by type II epithelial cells. These findings suggested that LGL1 also has a role in alveolarization. To test this hypothesis, we assessed postnatal LGL1 expression in two O<sub>2</sub> toxicity rat models of BPD. Reduced levels of LGL1 mRNA (northern analysis, n=2) and lg11 protein (immunohistochemistry (IHC), n=3) were observed in a rat model of BPD generated by exposing neonatal rats to 60% oxygen for 7, 10 or 14 days (n=4 litters/group). A second rat model was generated by exposing neonatal rats to 95% O<sub>2</sub> for 7 days. Exposure of these rats to air for 1, 2 or 3 weeks allowed us to study the expression of LGL1 both following hyperoxia and during the recovery period of accelerated growth in air, using IHC (n=3). Lg11 protein levels are greatly reduced after one week exposure to 95% O<sub>2</sub>, but were gradually increased to normal levels after 3 weeks of air exposure (n=2/group). Controls were exposed to air for the same time periods for both animal models. We speculate that deficiency of lg11 contributes to the arrested alveolar partitioning observed in BPD and that recovery is associated with normalization of lg11 levels. These findings support a role for LGL1 in the normal process of alveolarization.

**Mutations in wing 2 of the winged-helix transcription factor FOXC1 disrupt transactivation ability.** *T.C. Murphy, R.A. Saleem, M. Yu, M.A. Walter.* Ophthalmology/Med Genetics, Univ Alberta, Edmonton, AB, Canada.

*FOXC1*, mapping to chromosome 6p25, is a developmentally important winged-helix transcription factor characterized by the presence of the forkhead domain (FHD). The FHD is composed of a helix-turn-helix core of three-helix bundle, flanked by two loops or wings. Wing 1 is stabilized by a  $\alpha$ -sheet and makes contacts with the DNA while the function of wing 2 is largely unknown. Mutations in *FOXC1* lead to aberrant development of the anterior segment of the eye and increase the risk of early onset glaucoma. We have recently identified a novel mutation in wing 2 of the *FOXC1* forkhead domain (G165R). Together with the recently described M161K mutation in wing 2 we have investigated the effects of these *FOXC1* mutations on the ability of the *FOXC1* protein to bind DNA and activate gene expression. Site-directed mutagenesis was used to introduce these missense mutations into the *FOXC1* cDNA. Epitope-tagged *FOXC1* constructs were expressed in COS-7 cells, producing products of the correct size by Western analysis. The effect of each wing 2 mutation on *FOXC1*-DNA interactions was tested by electrophoretic mobility shift assays (EMSAs), while the effects of these missense mutations on the transactivation ability of *FOXC1* were tested using a dual luciferase reporter assay. Both the missense mutations localize to the nucleus at wildtype levels and EMSAs have revealed that *FOXC1* carrying M161K or G165R is still able to bind an in vitro derived *FOXC1* binding site at wildtype *FOXC1* levels. Interestingly, these mutations demonstrate reduced transactivation of a luciferase reporter gene in comparison to wild type *FOXC1*. Further analyses of the effects of these missense mutations on *FOXC1* binding specificity and the nature of the transactivation disruption are underway. These experiments show that these missense mutations in *FOXC1* do not perturb *FOXC1*-DNA interactions, but do disrupt the transactivation potential of *FOXC1*. The analyses of these two *FOXC1* missense mutations provide an understanding of how the wing 2 region of the forkhead domain is involved in *FOXC1* function. These analyses will also help define critical thresholds of *FOXC1* function.

**Gene expression profiles of human craniofacial structures in early embryogenesis.** *Y. Korshunova<sup>1</sup>, D. Messina<sup>1</sup>, R. Tidwell<sup>1</sup>, C. Helms<sup>1</sup>, M. Lovett<sup>1</sup>, T. Attie-Bitach<sup>2</sup>, J. Auge<sup>2</sup>, S. Audollent<sup>2</sup>, M. Vekemans<sup>2</sup>, J. Cai<sup>3</sup>, D. Ash<sup>3</sup>, E.W. Jabs<sup>3</sup>.* 1) Washington University, St. Louis, MO; 2) Hospital Necker Enfants Malades, Paris; 3) The John Hopkins University, Baltimore, MD.

Craniofacial defects are among the most common congenital abnormalities in humans. More than 20% of these defects appear to have a genetic component, but we know very little about the genetic programming of the complex structures that make up the human face. We have conducted the largest expression study to date on human embryonic structures that give rise to the head and face between the 4th and 8th weeks of human gestation. We employed four overlapping methodologies (EST generation from cDNA libraries, microSAGE, Affymetrix Genechips and microarray analysis) to derive a detailed picture of gene expression changes across 24 microdissected structures. Comparison of Genechip data with SAGE tag frequencies indicated that, in general, the results from both methods were highly consistent. Likewise, hierarchical clustering of expression data from over 50 separate Genechip experiments revealed that duplicate hybridizations from single dissected structures were highly reproducible. Self organizing maps were used to cluster expression data from all 24 tissues and resulted in the identification of 21 clusters of genes with similar expression patterns. These 21 clusters comprised a total of 405 genes that significantly vary during craniofacial development. In addition to this analysis, we used genes known to be involved in craniofacial development (such as EYA1, MSX2 and TWIST) as the basis of expression clustering. For example, clustering around MSX2 revealed coordinate changes in FoxG1B and a the gene encoding craniofacial development protein1. Likewise, clustering on the basis of EYA1 expression revealed two additional genes that may be important in craniofacial development: FOXF1 and MEOX2. Our very large set of >500,000 data points provides the base line for the identification of important pathways and for many future investigations of the effects of gene disruptions and environmental effects upon craniofacial development.

**COP9 signalosome subunit 3 (*Csn3*) is essential for maintenance of cell proliferation in the mouse embryonic epiblast.** J. Yan<sup>1</sup>, K. Walz<sup>1</sup>, H. Nakamura<sup>1</sup>, S. Carattini-Rivera<sup>1</sup>, Q. Zhao<sup>1</sup>, H. Vogel<sup>3</sup>, N. Wei<sup>4</sup>, M.J. Justice<sup>1</sup>, A. Bradley<sup>5</sup>, J.R. Lupski<sup>1, 2</sup>. 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 3) Dept Pathology, Stanford University, CA; 4) Dept molecular, cellular, and developmental biology, Yale University, CT; 5) Sanger Centre, Cambridge, UK.

*Csn3*(*Cops3*) is located in the mouse chromosome 11 region syntenic to the common deletion interval for the Smith-Magenis Syndrome (SMS), a contiguous gene deletion syndrome manifested by anatomical and multi-developmental abnormalities and associated with a chromosome 17 deletion - del(17)(p11.2p11.2). It encodes the third subunit of the COP9 signalosome (CSN), that is an eight-subunit protein complex originally identified as a repressor of light mediated development in plants; mutants of this complex are lethal after the seedling stage. Disruption of two of the homologous subunits in *Drosophila* caused lethality at the late larval or pupal stages. To investigate CSN function in mammals, and its potential role in the SMS phenotype, we disrupted the murine *Csn3* gene in three independent ways using insertional vectors including constructing an ~ 3 Mb inversion balancer chromosome. The heterozygous mice appeared normal, although the protein level was reduced, and viable homozygous *Csn3* <sup>-/-</sup> mice were not obtained. Detailed analyses of 5.5 days postcoitum (dpc) to 11.5 dpc embryos revealed that homozygous *Csn3* <sup>-/-</sup> embryos arrested after 5.5 dpc and resorbed by 8.5 dpc. Mutant embryos form an abnormal egg cylinder which does not gastrulate. They have a reduced number of epiblast cells, mainly due to increased cell death. In the *Csn3* <sup>-/-</sup> mice, subunit 8 of the COP9 complex was not detected by immunohistochemical techniques suggesting the absence of *Csn3* may disrupt the entire COP9 complex. Therefore, *Csn3* is important for maintaining the integrity of COP9 signalosome and is crucial to the development of the postimplantation embryo in mice.

***Dby* expression and germ cell development.** *Q.P. Vong*<sup>1</sup>, *S.M. Wu*<sup>1</sup>, *M. Dym*<sup>2</sup>, *O.M. Rennert*<sup>1</sup>, *W.Y. Chan*<sup>1,2,3</sup>. 1) NICHD, National Institutes of Health, Bethesda, MD; 2) Department of Cell Biology, Georgetown University, Washington, DC; 3) Department of Pediatrics, Georgetown University, Washington, DC.

In mouse and human, deletion of the *AZFa* region of Y chromosome has been linked to early failure of spermatogenesis and consequent sterility. Molecular analysis identified six genes lying in that region: *Usp9y*, *Dby*, *Uty*, *Eif2s3y*, *Smcy*, and *Ube1y1*; all have closely similar X-encoded homologs. This interval is associated with the *Spy* spermatogenesis phenotype. Patients with deletion of *AZFa* frequently show Sertoli Cell Only syndrome; either low levels or no germ cells are present in seminiferous tubules. Of the Y-encoded genes which mediate the function of the Y chromosome in spermatogonial proliferation, *Dby* has been considered a strong candidate. Early studies using an indiscriminative probe showed that *Dby* was expressed primarily in testicular somatic cells. This observation implicated a regulatory role for *Dby* in spermatogonial proliferation during spermatogenesis. To understand the genetic regulation of spermatogenesis, we have profiled the transcriptome of type A spermatogonia (SgA) of the mouse using Serial Analysis of Gene Expression (SAGE) and found *Dby* expression in these cells. The full-length *Dby* gene was cloned from the same cell type. In mouse, as in human, the *Dby* gene gives rise to two alternative transcripts that differ only in the length of 3'untranslated region due to distinct polyadenylation signals. The long form was present in non-testicular tissues while the short form was found only in the testis. Both forms were shown to be present in SgA by molecular cloning and DNA sequencing. It is likely that the *Dby* signal previously observed in testicular somatic cells was contributed by the long transcript. Besides these two variants, two other types of alternatively spliced transcripts containing non-overlapping sequences of 48 and 120 nucleotides respectively, were found in SgA. Differential expression of these *Dby* variants in germ cells at different stages of spermatogenesis is being examined. The presence of *Dby* in SgA indicates that *Dby* has an intrinsic role in germ cell development and may explain the spermatogenic arrest observed in *AZFa* deletion patients.

**USING SAGE TO CONFIRM A TELOMERASE IMMORTALIZED SPERMATOGONIAL CELL LINE AS AN EXPERIMENTAL MODEL.** *N. Tayebi*<sup>1</sup>, *LX. feng*<sup>2</sup>, *V. Baxendale*<sup>1</sup>, *SM. Wu*<sup>1</sup>, *ALY. Pang*<sup>1</sup>, *M. Dym*<sup>2</sup>, *WY. Chan*<sup>1,2,3</sup>, *OM. Rennert*<sup>1</sup>. 1) Laboratory of Clinical Genomic, NICHD, NIH, Bethesda, MD; 2) Department of Cell Biology Georgetown University, Washington, DC; 3) Department of Pediatrics Georgetown University, Washington, DC.

The renewal and differentiation of spermatogonial stem cells into spermatozoa is a complex and continuous process. We are interested in identifying the genes and mechanisms of transcriptional control during spermatogenesis. To ascertain whether a Spermatogonial Cell Line (SCL) immortalized with mouse TERT (Feng et al, Science 2002) is an appropriate representation of Type A Spermatogonia (TAS) *in vivo*, we compared the Serial Analysis of Gene Expression (SAGE) profile of the SCL with that of isolated TAS. An SCL SAGE library was constructed from total RNA and 2,304 independent library clones were sequenced. SAGE 2000 analysis software and the SAGEmap data base were used to allocate UniGene cluster IDs to the tags. A total of 48,485 tags were identified, of which 17,042 were unique. Singleton tags not shared by both libraries were excluded. Comparison of SAGE tags from both libraries showed that 4,264 tags were SCL specific and 2,018 tags were expressed in both libraries. As the total number of tags in the SCL (48,485) and TAS (110,872) libraries was different, the percent of total for each tag was determined in each library. The ratio of the percentage of a tag in the two libraries was used as an indicator of its relative expression. Ratios between 0.5-2.5 were chosen for further analysis. Several known genes essential for spermatogenesis were identified in both libraries, including several growth factors and their receptors, Pumilio 2, MAD1, MAD2, RNA binding proteins, transcription factors, and SRY. *Daz1* was down-regulated in the TAS. The most abundantly expressed tags in the SCL which are shared or known to be expressed in TAS are candidates for further study using RT-PCR and/or Real Time PCR. The number of shared SAGE tags and genes identified shows that the SCL can be used as an *in vitro* experimental model to identify genes and transcriptional control systems involved in spermatogenesis *in vivo*.

**Expression of the *PAX8*, *TITF1*, *FOXE1* and *TSHR* genes during human development: new insights on human thyroid and kidney development.** S. SURA<sup>1,2</sup>, J. AUGÉ<sup>3</sup>, G. MATTEI<sup>3</sup>, H. ETCHEVERS<sup>3</sup>, J. MARTINOVIC<sup>3</sup>, P. CZERNICHOV<sup>1</sup>, M. VEKEMANS<sup>3</sup>, M. POLAK<sup>1,2</sup>, T. ATTIE-BITACH<sup>3</sup>. 1) INSERM U-457 et Service d'endocrinologie pédiatrique Hopital Robert Debre, Paris, FRANCE; 2) INSERM E0363 et Service d'Endocrinologie pédiatrique, Hopital Necker, Paris, FRANCE; 3) Departement de Genetique et INSERM U-393, Hopital Necker, Paris, FRANCE.

Congenital hypothyroidism affects about 1 in 3800 newborns. In most cases, it is linked to a defect in thyroid ontogeny. Inactivation of the *PAX8*, *TITF1*, *FOXE1* and *TSHR* genes in patients or mice produce thyroid dysgenesis. To better understand the phenotypes associated with human mutations of these genes, we decided to study their expression patterns at different stages of human development. *In situ* hybridization studies using specific [<sup>35</sup>S]UTP labeled probes were performed on 3 embryonic (CS14, CS15, CS19) and 2 fetal (9 and 11 developmental weeks) stages. *PAX8* and *TITF1* are the first genes expressed in the median thyroid primordium. *FOXE1* is detected later and *TSHR* is expressed only when the thyroid migration is completed. Interestingly, the *PAX8* gene is also expressed in the fourth pharyngeal arch ectoderm and later in an inner cell population corresponding presumably to the ultimobranchial body. Furthermore, the *PAX8* gene is expressed in ureteric bud and some of its derivatives in developing kidney. Finally, *FOXE1* is expressed in human thymus. In conclusion the expression patterns described here are distinct from those reported in animal models and correlate with the congenital anomalies observed in humans.

**Animal models for Amish lethal microcephaly.** *M.J. Lindhurst, T. Blake, A. Chen, P.P. Liu, L.G. Biesecker.* National Human Genome Research Institute, NIH, Bethesda, MD.

Amish lethal microcephaly (MCPHA) is an autosomal recessive disorder comprising severe microcephaly, a congenital malformation of the brain, increased urinary  $\alpha$ -ketoglutarate, and premature death. Previously we reported the discovery of a single nucleotide substitution in the *SLC25A19* gene encoding a mitochondrial membrane deoxynucleotide carrier, DNC, which segregates with the disease in affected individuals and alters an amino acid highly conserved in similar proteins. Functional analysis of the mutant protein revealed that normal transport activity was lost. We hypothesize that insufficient transport of dNTPs into mitochondria in the developing central nervous system interferes with mitochondrial DNA synthesis, causing abnormal brain growth and development. Zebrafish knockdown experiments confirm that this protein is necessary for normal development as two morpholinos targeted to DNC caused a range of CNS and cranial malformations in a dose-dependent fashion. The rostral portion of mutant embryos was smaller and more opaque than controls suggesting that tissues in this region have become necrotic. Histological examination of serial sections revealed the presence of a large vacuole in the dorsal region of the head. *In situ* hybridizations with *Wnt1* and *Krox20* showed expression patterns in mutant embryos that resembled those of earlier timepoints than those of control embryos indicating that DNC is necessary for proper formation of the zebrafish brain. We have also created a DNC knockout mouse and have begun breeding experiments to study the affects of the mutation in the homozygous state. These results show the critical relationship between energy metabolism and brain growth and development as well as suggest that perturbation of energy metabolism may explain other types of brain growth disorders.

**Evaluation of Zebrafish as a Model Organism for the Human Bardet-Biedl Syndrome.** *H.-j. Yen*<sup>1,2</sup>, *K. Mykytyn*<sup>1,2</sup>, *D.Y. Nishimura*<sup>1</sup>, *R.F. Mullins*<sup>3</sup>, *C.C. Searby*<sup>1,2</sup>, *T.A. Westfall*<sup>4</sup>, *D.C. Slusarski*<sup>4</sup>, *V.C. Sheffield*<sup>1,2</sup>. 1) Dept. of Pediatric, Division of Medical Genetics, University of Iowa, Iowa City, IA; 2) Howard Hughes Medical Institute; 3) Dept. of Ophthalmology, University of Iowa, Iowa City, IA; 4) Dept. of Biological Sciences, University of Iowa, Iowa City, IA.

Bardet-Biedl syndrome (BBS) consists of the primary features of obesity, retinitis pigmentosa, polydactyly, renal and cardiac malformations, mental retardation and hypogonadism. Patients with BBS also have increased risk of developing hypertension and diabetes mellitus. BBS is an autosomal recessive disease that exhibits locus heterogeneity and is known to map to seven human loci: 11q13 (*BBS1*), 16q22 (*BBS2*), 3p13 (*BBS3*), 15q21 (*BBS4*), 2q31 (*BBS5*), 20p12 (*BBS6*) and 4q27 (*BBS7*). To date, 5 BBS genes (*BBS1*, *BBS2*, *BBS4*, *BBS6* and *BBS7*) have been identified. Complex inheritance of BBS has been proposed. In order to investigate complex inheritance of the disorder, we have used zebrafish as a model organism. We have identified putative zebrafish orthologs using bioinformatic approaches. Comparisons of the amino acid sequences of zebrafish and human BBS gene products show high homology (on average 70% identity and 80% similarity). We performed whole mount *in situ* hybridization in embryos between 0 to 24 hours post fertilization and determined that zBBS genes are ubiquitously expressed. We performed z*BBS4* morpholino antisense oligo gene knockdown experiments and observed retinal abnormalities in a dose-dependent manner. Furthermore, we performed functional vision assays and confirmed that the retinal abnormalities correlate with blindness. Histological analyses indicate that disrupting the expression of z*BBS4* results in ocular abnormalities including a failure of the retinal cell layers to form properly. In addition, we performed double knockdown experiments and preliminary results indicate that *BBS2* and *BBS6* interact genetically. This work supports the use of zebrafish as a model for the molecular pathogenesis of BBS.

**Microarray and functional analyses of primordial follicle formation and the etiology of premature ovarian failure.** *C. Ottolenghi<sup>1,3</sup>, M. Uda<sup>1,2</sup>, L. Herrera<sup>1</sup>, E. Garcia<sup>1</sup>, A. Forabosco<sup>3</sup>, D. Schlessinger<sup>1</sup>, L. Crisponi<sup>2</sup>, M. Deiana<sup>2</sup>, G. Pilia<sup>2</sup>.* 1) Laboratory of Genetics, GRC, NIA/NIH, IRP, Baltimore, MD; 2) Istituto di Ricerca sulle Talassemie ed Anemie Mediterranee CNR, and Dipartimento di Scienze Biomediche e Biotecnologie, Ospedale Regionale per le Microcitemie, University of Cagliari, Cagliari, Italy; 3) Genetica Medica, Department Mother and Child, University of Modena, Italy.

The stage of primordial follicle formation is critical for female fertility, correlated with selection of functional oocytes from a far larger fetal pool. In the mouse, this stage is restricted to the perinatal period, providing an opportunity to investigate critical factors. As complementary approaches, we have first applied expression profiling of gene expression in microarrays, and have found that over one hundred genes, including a number of transcription factors and novel transcripts, are differentially expressed between the first and later phases of follicle formation. Second, we have knocked out the FOXL2 gene (mutations in which are a cause of premature ovarian failure in humans), and are currently assaying its impact on fertility. Third, we are characterizing gene expression profiles in an embryonic stem cell-based in vitro model for the first stages of ovarian follicle development. These approaches provide a framework to identify genes and functions specifically involved in setting the level of functional oocytes.

**Identifying New Sex-Determining Loci Using a Congenic Mouse Model.** *G. Nikolova*<sup>1</sup>, *E. Vilain*<sup>1,2,3</sup>. 1) Human Genetics, UCLA, Los Angeles, CA; 2) Dept of Pediatrics, UCLA, Los Angeles, CA; 3) Dept of Urology, UCLA, Los Angeles, CA.

In mice as well as humans, sex is determined by the presence or absence of Sry, a male specific gene found on the Y chromosome. Unlike humans however, the mouse Sry protein sequence differs among individual strains, especially between members of the *Mus musculus musculus*, and *domesticus* subspecies. Introducing a number of different *domesticus* Y chromosomes onto the inbred *musculus* C57Bl/6J genetic background results in varying degrees of XY sex reversal despite the fact that sexual development proceeds normally in the parental *domesticus* strain. Further investigations of the phenomenon have confirmed that the variability in Sry together with interacting factors from C57Bl/6J contribute to it, but neither specific Sry residues, nor C57Bl/6J polymorphisms have been implicated. Since many other *musculus* inbred strains, such as 129S1/Sv, are not subject to this form of sex reversal, mouse genetics can be used to investigate the C57Bl/6J components affecting the phenotype. 129S1/Sv males carrying a Y chromosome from the *domesticus* subgroup Poschiavinus, were mated with C57Bl/6J females, followed by repeated backcrossing of male offspring to C57Bl/6J females for 13 generations. Continuous selection for fully masculinized males resulted in generating a C57Bl/6J-XY<sup>POS</sup> congenic strain homozygous for the 129S1/Sv element(s) conferring protection against sex reversal. In order to identify the location of the congenic fragment(s) we have genotyped autosomal microsatellite markers spaced 15-20 cM apart in these C57Bl/6J-XY<sup>POS</sup> and control C57Bl/6J mice. Each inbred mouse strain has a characteristic PCR product size for a given marker, which can be used to distinguish the genetic background of origin at that locus. Markers for which the congenic does not match C57Bl/6J may thus potentially identify chromosomal segments containing genes involved in sex determination. Further analysis of such regions is being performed by creating a denser marker map, as well as by evaluating the sequence and expression pattern of candidate genes.

**Wnt4 signaling inhibits steroidogenesis by antagonizing Sf-1 and  $\beta$ -catenin.** *E. Vilain*<sup>1,2,3</sup>, *J.H.-C. Shen*<sup>4</sup>, *R. Olaso*<sup>4</sup>, *H.A. Ingraham*<sup>4</sup>, *B.K. Jordan*<sup>1</sup>. 1) Dept of Human Genetics, UCLA Sch of Medicine, Los Angeles, CA; 2) Dept of Pediatrics, UCLA Sch of Medicine, Los Angeles, CA; 3) Dept of Urology, UCLA Sch of Medicine, Los Angeles, CA; 4) Dept of Physiology, UCSF Sch of Medicine, San Francisco, CA.

In mammals, the genes of the sexual development pathway direct the cells of the bipotential gonad to organize in sexually-dimorphic ways including production of steroidogenic hormones. Genetic studies in mice have suggested that Wnt4 signaling suppresses the expression of enzymes controlling the biosynthesis of male hormones, thus effectively blocking their production in the female embryo. Recently, we identified an XY intersex patient carrying a duplication of the *WNT4* locus and demonstrated that overexpression of *WNT4* was the likely cause of this patient's feminization. To test this hypothesis, we generated a transgenic mouse model overexpressing *WNT4*. Although sexual development of transgenic females was unaffected by the increase in *WNT4* expression, transgenic males exhibited abnormal testis development with fewer germ cells and disorganization of the vascular system. Although a complete male-to-female sex reversal was not observed, testosterone levels were markedly reduced in transgenic males. This Wnt4-mediated repression of steroidogenesis was recapitulated in both Leydig and adrenocortical cell lines co-cultured with cells overexpressing *Wnt4*, which exhibited reduced progesterone secretion and 3-hydroxysteroid dehydrogenase activity, respectively. Further *in vitro* studies revealed that *Wnt4* antagonizes the functional synergy observed between  $\beta$ -catenin, the major effector of the Wnt signaling pathway, and Sf-1, the major regulatory factor of steroidogenesis. In addition, chromatin immunoprecipitation studies suggest that *Wnt4* attenuates recruitment of  $\beta$ -catenin to an Sf-1-target gene promoter. Taken together, these data provide evidence that *Wnt4* acts as an anti-male factor in sexual development. We propose a model in which Wnt4 signaling antagonizes steroid production by disrupting recruitment of  $\beta$ -catenin and Sf-1 to binding sites present in multiple steroidogenic target genes.

**Correlations between DNA, FMRP, and mRNA levels and ADOS-G and ADI-R scores in patients with Fragile X Syndrome.** *K. Herman<sup>1</sup>, S. Nowicki<sup>1</sup>, F. Tassone<sup>3</sup>, K. Koldewyn<sup>1</sup>, S. Bacalman<sup>1</sup>, S. Jacquemont<sup>1</sup>, I. Barboto<sup>1</sup>, R. Levine<sup>2</sup>, P. Hagerman<sup>3</sup>, R. Hagerman<sup>1</sup>.* 1) M.I.N.D. Institute, UC Davis Health System, Sacramento, CA; 2) Department of Mathematics and Statistics, San Diego State University, San Diego, CA; 3) Department of Biological Chemistry, UC Davis, Davis, CA.

Several papers have looked at the involvement of CGG repeat number, methylation status, and FMRP levels on the clinical phenotype of Fragile X Syndrome. However, mRNA levels have not been studied with regard to the Fragile X phenotype. In older male carriers of the premutation, we have documented the presence of a Fragile X Associated Tremor/Ataxia Syndrome (FXTAS), which is thought to be related to a toxic effect of elevated mRNA levels. In addition, we have seen autism in children with the premutation that may also be related to elevated message, low FMRP, or additional genetic effects. Since 33 percent of Fragile X patients have autistic spectrum disorders, we decided to examine the relationship between molecular measures and autism diagnostic measures (ADOS-G and ADI-R). We studied 66 patients with either Fragile X Syndrome (58 patients) or affected premutation carriers (8 patients), both with (31 patients) and without (35 patients) autistic spectrum disorders.

Neither ADI nor ADOS scores correlated with CGG repeat number. FMRP levels correlated only marginally with ADOS and ADI scores ( $p=0.08$ ). ADOS total scores had a strong correlation with mRNA levels ( $p=0.018$ ), even with controlling for IQ ( $p=0.029$ ). When the ADOS subscores were analyzed, it was found that mRNA levels correlated with the ADOS Communication scores at a significant level ( $p=0.0063$ ), but did not correlate with the ADOS Social scores ( $p=0.13$ ). In addition, the results showed a U shaped distribution, with both high and low mRNA levels correlating with high ADOS Communication scores.

The presence of autism in Fragile X Syndrome may be related to a number of factors including background gene effects, lowered FMRP, and elevated mRNA. These results suggest that elevated message may have the strongest effect, particularly on communication.

**Genome wide expression profile in trophoblastic disease.** *S. Jasani*<sup>1</sup>, *Y. Lin*<sup>2</sup>, *E. Feingold*<sup>2</sup>, *U. Surti*<sup>1</sup>. 1) Pathology, University of Pittsburgh, Pittsburgh, PA; 2) Biostatistics, GSPH, University of Pittsburgh, Pittsburgh, PA.

Affymetrix U113A oligonucleotide microarray chips were used to obtain genome wide expression profiles of fibroblasts from 3 androgenetic complete hydatidiform moles(CHM-PP), 3 normal biparental chorionic villus samples (NML-MP), 5 triploid partial hydatidiform moles(PHM-MPP) and 3 nonmolar triploids(NMT-MMP). The CEL files obtained after hybridization were used to scale and normalize the data using dCHIP software. Modified t-test that uses empirical Bayesian methods was used to obtain a list of most differentially expressed genes between NML-MP and CHM-PP as well as PHM-MPP and NMT-MMP. In addition, expression profiles of 2 choriocarcinoma cell lines were also obtained. Several genes constituting cell growth pathways such as the MAP kinase pathway, and/or cell death pathways are differentially expressed. Due to the androgenetic nature of a complete mole, special emphasis was placed on the expression of imprinted genes, most of which are expressed in the placenta and are important to growth and regulation of the fetus and placenta. Two important cell growth and regulatory pathways were explored in detail. These are the IGFII pathway and the TGF pathway. Several genes involved in these pathways are differentially expressed. IGFII, one of the major imprinted, placental growth promoters, is upregulated in complete moles, along with IRS-1, while IGF2R is downregulated, indicating an increased signaling through either the insulin or IGFIR, contributing to the increased size of the placenta in complete moles. This pathway is interconnected to the TGF pathway, which also has an independent function in the placenta. TIMP1 and PAII are genes directly controlled by TGF, and that are downregulated in complete moles. PEG1/MEST, PEG3, PEG10, SGCE, SNRPN, NOEY2 and TSSC3 were among differentially expressed genes in CHM-PP when compared to NML-MP as expected. Most of these genes were also among differentially expressed when PHM-MPP results were compared to NMT-MMP. These and other differentially expressed genes observed contribute to the peculiar phenotypes of complete mole, as well as type-1(large placenta) and type-2(small placenta, macrocephaly) triploids.

**Genome-wide gene expression comparison of forelimb versus hindlimb development by microarray technique.** S. Shou<sup>1, 2</sup>, H.S. Stadler<sup>1, 2</sup>, *Genomics, Development*. 1) Research Center, Shriners Hospital, 3101 SW Sam Jackson Park Road, Portland, OR 97239; 2) Oregon Health and Science University, Department of Molecular & Medical Genetics, 3181 SW Sam Jackson Park Road, Portland, OR 97239.

Analysis of gene expression profiles in limb tissues is an important approach to elucidate molecular mechanisms of limb development. The expression profiles of the developing forelimb and hindlimb were assessed using the Affymetrix mouse expression 430A/B GeneChips and total RNAs isolated from E 12.5 forelimb or hindlimb autopods. A transcriptome of the expressed genes in the autopod was generated detecting expression of approximately 24907 genes in the forelimb or hindlimb samples, 14748 genes representing 65% of the probe sets on MOE-430A GeneChip, and 10159 genes or ESTs representing 45% of the probe sets on MOE-430B GeneChip. Among the genes detected in the forelimb and hindlimb samples, 4.44% or 1105 genes and ESTs were differentially expressed between the forelimb and hindlimb samples as measured by the Affymetrix MAS 5.0 algorithm ( $|\text{Changed P value}| < .0006$ ,  $|\log_2 \text{Ratio}| > 0.2$ ). A greater than 2-fold expression difference between forelimb and hindlimb samples ( $|\log_2 \text{Ratio}| > 1$ ,  $|\text{Changed P}| < 0.006$ ) was detected for 136 genes or ESTs including *Tbx4*, *Tbx5*, and *Pitx1* which were previously shown by SAGE to be differentially expressed in the forelimb or hindlimb (Margulies 2001). Similarly, genes such as members of the BMP, FGF, EPHA, WNT, CASPASE, HSP, and HOX gene families were also found to be highly expressed or differentially expressed in developing autopods, suggesting that the differential expression and differential combinations of gene expression in the developing limbs contribute to differences in forelimb versus hindlimb patterning required for morphological diversification of these structures. Key word: Microarray, Embryo, Limb, Autopod, Gene expression, Development.

**CONGENITAL PULMONARY LYMPHANGIECTASIA AND CONGENITAL CHYLOTHORAX ARE CLOSELY RELATED ENTITIES.** *C. Bellini<sup>1</sup>, F. Boccardo<sup>2</sup>, C. Campisi<sup>2</sup>, G. Taddei<sup>3</sup>, E. Bonioli<sup>1</sup>, M. Baldi<sup>1</sup>, C. Arioni<sup>1</sup>, R. Sacchi<sup>1</sup>, S. Ciotti<sup>1</sup>, G. Serra<sup>1</sup>.* 1) Servizio Patologia Neonatale, Gaslini Inst, Univ, Genoa, Italy; 2) Dipartimento Scienze Chirurgiche, Univ, Genoa, Italy; 3) Dipartimento Medicina Interna Univ, Genoa, Italy.

**Purpose.** To evaluate correlation between congenital pulmonary lymphangiectasia (CPL) and congenital chylothorax (CC). Congenital pulmonary lymphangiectasia (CPL) is a rare developmental disorder of the pulmonary lymphatics. CPL is characterized by numerous dilated lymphatic channels in the sub-pleural connective tissue, in the interlobular septa, and along bronchovascular axes. Chylothorax is defined as an accumulation of chyle in the pleural space. The term congenital chylothorax (CC) is usually limited to congenital abnormalities of the lymphatic system, and CC is present at birth. **Methods.** We report on three infants with congenital chylothorax (CC) and congenital pulmonary lymphangiectasia (CPL). **Results.** Clinical course, imaging and lymphoscintigraphic studies demonstrated the occurrence of CPL and CC, as well as lymphatic dysplasia. **Conclusion.** CPL and CC are often described as separate entities. This is mainly due to the low number of existing reports on CPL that include biochemical and cytological analysis of the pleural effusion, as well as to the limited number of cases of both CPL and CC, whose diagnoses had been prevented by lack of adequate diagnostic studies. CPL appears to be a constant pathological finding in CC. This study suggests that CC and CPL are strongly correlated entities and that the dysplasia of the lymphatic system results in a pulmonary lymphatic obstruction sequence. The initial, microscopic dilatation of the lymph channels leads to progressive weeping of lymphatics and, consequently, to pleural effusion. Non-Immune Hydrops Fetalis (NIHF) may be the final consequence of impaired systemic venous return and may help to explain pleural-pulmonary involvement of this generalized lymph-vessel malformation syndrome. This report focuses on CPL-CC-NIHF in neonates. On the basis of radiological and lymphoscintigraphic evaluation, it is suggested that there is a strong correlation among these entities.

**CONGENITAL CHYLOTHORAX IN NEWBORNS.** *F. Boccardo*<sup>1</sup>, *C. Bellini*<sup>2</sup>, *C. Arioni*<sup>2</sup>, *M. Baldi*<sup>2</sup>, *R. Sacchi*<sup>2</sup>, *S. Ciotti*<sup>2</sup>, *E. Bonioli*<sup>2</sup>, *C. Campisi*<sup>1</sup>, *G. Serra*<sup>2</sup>. 1) Dip Scienze Chirurgiche, Univ Genoa, Italy, Genoa, Genoa, Italy; 2) Servizio Patologia Neonatale, Univ Genoa, Gaslini Inst, Genoa, Italy.

Chylothorax is defined as an accumulation of chyle in the pleural space. The term congenital chylothorax (CC) is usually reserved for congenital abnormalities of the lymphatic system. Establishing the diagnosis and selecting the most appropriate therapy may be challenging. We report our experience in managing chylothorax of the neonatal age and discuss various congenital malformed condition that can be associated with chylothorax. Four cases of premature newborns born with CC and various degree of non-immune hydrops fetalis have been observed. CC is a rare cause of respiratory distress in the newborn and is the most common form of pleural effusion in the neonatal period. The real incidence of congenital chylothorax is not known, ranging from 1:1,000 to 1:15,000 pregnancies. The actual incidence is probably higher, and intrauterine fetal death as well as stillbirth might be underestimated. Lymphangiomas and lymphangiectasia are the two major abnormalities of lymphatic development that cause chylothorax. Abnormal prominence of subpleural and interlobular lymphatics typically occur in pulmonary lymphangiectasia. Obstructed, dilated lymphatics are unable to drain lymph appropriately (though an obstruction site has yet to be elucidated) and contribute to a non-physiologic stiffening of lung parenchyma. Thus, in some cases, chylothorax may occur. The exact mechanism through which chylothorax develops to a more generalized hydrops is poorly understood. Prognosis is unpredictable. Overall mortality for congenital chylothorax has been reported ranging 50-95%. The presence of hydrops fetalis has significant prognostic implications. (However, non immune hydrops caused by chylothorax may carry a better prognosis than non immune hydrops in general. The frequency of spontaneous resolution is unknown, occurring both antenatally and postnatally.

**Cholesterol supplementation does not appear to improve developmental progress in Smith-Lemli-Opitz syndrome.** *D.M. Sikora, M. Ruggiero, K. Pettit-Kekel, L.S. Merkens, W.E. Connor, R.D. Steiner.* Depts of Pediatrics & Medical and Molecular Genetics, Child Development & Rehabilitation Center, Oregon Health & Science Univ, Portland, OR.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive multiple malformation/mental retardation syndrome. Clinical manifestations, including motor delay with hypo/hypertonia, language delay, oral-motor dysfunction, and mental retardation, are caused by impaired conversion of 7-DHC to cholesterol in the final step of cholesterol biosynthesis, resulting in cholesterol deficiency and elevated and potentially toxic accumulation of 7-DHC throughout the body and brain.

There is anecdotal evidence from several investigators suggesting that cholesterol supplementation improves developmental progress and reduces atypical behavior in children with SLOS; however, none of these studies reported scores from standardized instruments to document changes over time. The purpose of the present study was to provide longitudinal data on the developmental progress of 14 children with SLOS.

Eight females and six males with SLOS participate in a prospective longitudinal study assessing developmental progress during cholesterol supplementation. All subjects completed at least two developmental assessments, scheduled at least one year apart, over a 6 year period. The duration of cholesterol supplementation averaged 8 months prior to the first assessment. Cholesterol supplementation has been continuous throughout participation in the study.

Scores from standardized tests in the areas of motor, cognitive, and adaptive skill development did not improve over time for these subjects, based on statistical analyses using a repeated measures design. In addition, baseline cholesterol levels, rather than age when supplementation began or increase in cholesterol levels, best predicted developmental outcome using multiple t-tests. These results suggest that cholesterol supplementation in its current form does not improve the developmental progress of children with SLOS.

**Phenotypic characterization and positional cloning of two recessive ENU-induced mouse models of cleft palate.**

*B.C. Bjork*<sup>1</sup>, *B.J. Herron*<sup>2</sup>, *A.R. Vieira*<sup>3</sup>, *J.C. Murray*<sup>3</sup>, *D.R. Beier*<sup>1</sup>. 1) Div. Genetics, Brigham & Women's Hospital, Boston, MA; 2) Wadsworth Genomics Institute, Albany, NY; 3) Dept. Pediatrics, The Univ. of Iowa, Iowa City, IA.

We are screening E18.5 embryos from ENU-treated mice for recessive phenotypes that resemble human congenital disorders. Among the large spectrum of phenotypic abnormalities we have identified are a significant number of cleft secondary palate. Non-syndromic orofacial clefting occurs in about 1 in 1000 live births. Many known mutant mouse phenotypes include orofacial clefts, but the best models for human clefting are those with isolated clefts. The *cleft palate only 1* (*cpo1*; isolated cleft secondary palate) and *curly tail/cleft palate* (*ctcp*; cleft secondary palate and/or a curly or kinked tail) mutations are excellent models of cleft secondary palate. Genetic fine-mapping defined critical regions of 500 kb on distal chr 4 and 3.5 Mb on proximal chr 3 for the *cpo1* and *ctcp* mutations, respectively.

We report the genetic localization, fine-mapping, developmental characterization and candidate gene screening for these ENU-induced recessive mutations. Most recently, we determined that *cpo1* mutant mice carry a mutation that affects splicing efficiency of a zinc finger transcription factor gene. We performed a histological evaluation of *cpo1* mutant embryos to identify a specific defect in palatal shelf elevation. RNA probes and polyclonal antibodies were generated to determine the developmental expression pattern of this zinc finger gene and its protein product and to begin to elucidate its function during craniofacial development, including identification of interacting proteins and downstream target genes. Finally, four potential etiologic missense mutations were identified in a screen of 100 individuals each from non-syndromic cleft populations from Iowa and the Philippines. These sequence variants have not yet been identified in control populations, thereby supporting a minor contribution by this gene to the complex etiology of non-syndromic clefting.

Program Nr: 946 from 2003 ASHG Annual Meeting

**Expression of Steroidogenic Acute Regulatory(StAR) Protein during Oocyte Apoptosis in the Gonadotropin-Stimulated Human Ovary.** *S.K. Kim<sup>1</sup>, H.W. Yang<sup>2</sup>, S.W. Bai<sup>1</sup>, K.H. Park<sup>1</sup>, D.J. Cho<sup>1</sup>, C.H. Song<sup>1</sup>.* 1) Dept. of OB/GYN College of MedicineYonsei Univ. CPO Box 8044 Seoul,Korea; 2) Dept.of OB/GYN College of MedicineUlji Univ. Seoul,Korea.

To determine the distribution and expression of steroid acute regulatory(StAR) protein in human oocyte and embryo in relation to apoptosis. Immuno-labelling and confocal microscopy were applied to examine the localization of StAR protein in human oocytes and embryos. Western blot analysis was also used for qualitative and quantitative assessment of StAR protein expression. There were lipid droplet accumulation in fragmented human oocytes and embryos. StAR protein (30 kDa) expression was detected in human oocytes and embryos. The level of StAR protein expression was lower in the fragmented group than the normal group. The present study provides evidence for involvement of StAR protein in the apoptosis of fragmented oocytes and embryos during in vitro fertilization (IVF) program as well as in the normal development of human oocytes and embryos.

**Vision defects and incomplete cone bipolar interneuron differentiation due to loss of function of the *Vsx1* homeobox gene.** *R.R. McInnes*<sup>1</sup>, *R.L. Chow*<sup>1</sup>, *B. Vogyi*<sup>3</sup>, *R.K. Szilard*<sup>1</sup>, *D. Ng*<sup>1</sup>, *C. McKerlie*<sup>2</sup>, *S.A. Bloomfield*<sup>3</sup>, *D.G. Birch*<sup>4</sup>. 1) Progs in Dev Biol, Genetics &; 2) Integrative Biology, The Research Institute, Hosp for Sick Children, Toronto, ON, Canada; 3) Depts of Ophthalmology and Physiology & Neuroscience, New York University School of Medicine, New York, NY, USA; 4) Retina Foundation of the Southwest, Dallas, TX, USA.

Bipolar interneurons of the vertebrate retina are essential for receiving and transmitting visual signals to retinal ganglion cells. One major class of bipolar interneurons, cone bipolar (CB) cells, are a molecularly and physiologically heterogeneous population that express the homeobox gene, *Vsx1*. *Vsx1* is a rapidly evolving *paired*-like homeo- and CVC-domain transcription factor whose homologues in vertebrates and worms play essential roles in interneuron development. In the retina, *Vsx1* is expressed in CB cells as they differentiate, suggesting it may play a critical role in CB cell formation. To examine this possibility, we targeted the *Vsx1* gene in mice. In *Vsx1*<sup>-/-</sup> mice, the specification, number and gross morphology of CB cells in the retina was normal, but great reductions were identified in the expression of at least four proteins (recoverin, NK3R, Neto1, and CaB5) characteristically expressed in OFF-CB cells. These defects in CB differentiation were accompanied by a reduced mixed rod-cone electroretinogram b-wave similar to that observed in human patients with dominant *VSX1* missense mutations (Heon et al. *HMG* 11, 1-8, 2002). This defect in visual transduction was also manifest by a general reduction in the light-evoked activity of OFF- but not ON-ganglion cells in the mesopic range. Importantly, *Vsx1*<sup>-/-</sup> mice had no corneal abnormalities, in contrast to humans carrying a single *VSX1* missense allele. Moreover, no expression of *Vsx1* was detected in the developing or mature mouse cornea, or adult human cornea. Our findings establish that *Vsx1* is essential for OFF-CB differentiation and visual signal transduction, and elucidate the basis of the abnormalities in visual transduction in humans with *VSX1* mutations. The pathogenesis of autosomal dominant human *VSX1*-associated corneal dystrophies, however, remains unclear.

**T cell clones as a tool for examining discrete FMR1 alleles in a common genetic background.** *D.H. Yasui<sup>1</sup>, F. Tassone<sup>1</sup>, P.K. Sahota<sup>1</sup>, A.K. Taylor<sup>2</sup>, R.J. Hagerman<sup>3</sup>, P.J. Hagerman<sup>1</sup>*. 1) Dept. of Medical Biochemistry, UC Davis School of Medicine, Davis, CA; 2) Kimball Genetics, Salt Lake City, UT; 3) MIND Institute, UC Davis School of Medicine, Sacramento, CA.

Southern and PCR analyses of whole blood from a high functioning, male, fragile X patient revealed genetic mosaicism in that 81% of *FMR1* alleles were in either the normal (5-54 CGG repeats) or premutation (55-200 CGG repeats) range and 19% were full mutation alleles (>200 CGG repeats). In order to examine individual *FMR1* alleles, T lymphocyte cloning was performed from peripheral blood mononuclear cells (PBMCs). Southern blot analysis showed that each T cell clone exhibited a discrete allele size that was stable throughout culture. Surprisingly, the preponderance (70%) of discrete *FMR1* alleles in the individual clones harbored full mutation alleles, with only 30% falling within the normal or premutation ranges. In contrast, previous studies in fibroblasts suggested that cells bearing full mutation *FMR1* alleles were negatively selected in vitro. Furthermore, 6% of the T cell clones contained intermediate *FMR1* alleles that were not detected in whole blood. Consistent with previous studies, quantitative RT-PCR analysis of RNA from individual T cell clones revealed that full mutation alleles produce virtually no *FMR1* transcripts while normal and premutation alleles produce normal or elevated levels of transcripts. These results suggest that: (1) there is no selection bias against cells with fully expanded *FMR1* alleles, (2) *FMR1* alleles are mitotically stable in adult tissues, (3) *FMR1* allele diversity in mosaic individuals is greater than what is revealed by Southern analysis of the pool of heterogeneous cell populations found in blood. T cell clones derived from \* mosaic patients are therefore a powerful tool for examining the molecular basis of fragile X syndrome.

**Narcolepsy: involvement of imprinted, differentially-methylated promotor haplotypes of vesicular monoamine transporter 2 (VMAT2).** *T. Drgon*<sup>1</sup>, *M. Takahashi*<sup>1</sup>, *D. Walther*<sup>1</sup>, *Z. Lin*<sup>1</sup>, *L. Lin*<sup>2</sup>, *M. Tafti*<sup>3</sup>, *M. Billiard*<sup>3</sup>, *M. Thorpy*<sup>5</sup>, *H. Temple*<sup>5</sup>, *J. Montplaisir*<sup>4</sup>, *E. Mignot*<sup>2</sup>, *G.R. Uhl*<sup>1</sup>. 1) Molecular Neurobiology, NIDA/NIH, Baltimore, MD; 2) Stanford Med. Ctr, CA; 3) Neurol. B, Montpellier, France; 4) Hopital du Sacre-Coeur, Montreal, Canada; 5) Montefiore Med. Ctr, New York, NY.

Narcolepsy is a familial sleep disorder with dysregulated REM sleep often accompanied by sleep paralysis and cataplexy. Since stimulants treat narcoleptic symptoms, genes implicated in stimulant actions including VMAT2 (SLC18A2) are candidates to harbor narcolepsy-predisposing variants. VMAT2 accumulates monoamine neurotransmitters into synaptic vesicles, while stimulants such as amphetamines release the same neurotransmitters from these vesicles and reduce the amounts released when neurons are depolarized. We now describe VMAT2 "B" haplotypes that are differentially transmitted from mothers to narcoleptic offspring in trio samples. Since these findings suggested imprinting, we examined methylation patterns of CpG sites located in VMAT2 5 flanking/promoter region. Allele-specific differences in methylation in the vicinity of specific transcription factor binding sites supported this mechanism. Maternally-inherited, imprinted VMAT2 haplotypes thus represent the second identified genetic contributor to vulnerability to this sleep disorder, providing effects more modest than those exerted by HLA haplotypes. The VMAT2 haplotypes identified in these studies are also likely to play pleiotropic roles in other disorders in which monoaminergic mechanisms play central roles.

**A *Drosophila* Model to Investigate MeCP2 Function and Interactions.** H. Cukier<sup>1</sup>, H.Y. Zoghbi<sup>1,2,3,4,5</sup>, J. Botas<sup>1,4,6</sup>.  
1) Human and Molecular Genetics; 2) Pediatrics; 3) Division of Neuroscience; 4) Program in Developmental Biology;  
5) Howard Hughes Medical Institute; 6) Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX.

The neurodevelopmental disorder Rett syndrome is caused by the loss of function of methyl-CpG-binding protein 2 (MeCP2), a protein implicated in chromatin remodeling. To gain insight about the *in vivo* function of MeCP2, we rationalized that a *Drosophila* model would be an ideal approach. *Drosophila* has proven to be an excellent organism to study *in vivo* function, interactions and modifiers of human disease genes. If MeCP2 can produce a phenotype in *Drosophila*, then large scale modifier screens can be performed to gain insight about the wild type protein and its *in vivo* interactions. Using the Gal4-UAS system, we have created eight independent lines which overexpress the full length human MeCP2. When these lines were crossed to flies carrying both elav-Gal4 (drives expression in neurons) and GMR-Gal4 (drives expression in the eye), all lines showed disorganized eyes. In addition, six of the lines demonstrated a variable crumpled wing phenotype and lethality. These stronger phenotypes correlated with higher levels of MeCP2 protein. To determine if these gain of function phenotypes require binding of the methyl-CpG-binding domain to *Drosophila* DNA or are due to interaction with the transcription repression domain, we are generating more transgenic strains using various alleles of MeCP2. Genetic screens are also currently being performed with the full length transgenic lines to identify modifiers and additional pathways in which MeCP2 may play a role. We identified one modifier that suppresses the eye- and wing-specific phenotypes. Further studies are ongoing to understand how this suppressor modulates MeCP2's function. Through investigation of MeCP2's molecular function in the *Drosophila* model system, we hope to gain insight into Rett syndrome pathogenesis.

**Targeted disruption of *Ube3a* alters the expression levels of *Ube3a-ATS* in *trans*.** M. Landers<sup>1</sup>, H. Glatt-Deeley<sup>1</sup>, J. Wagstaff<sup>2</sup>, M. Lalande<sup>1</sup>. 1) Dept of Genetics & Dev Biology, Univ of Connecticut Health Center, Farmington, CT 06030; 2) Dept of Pediatrics & Dept of Biochemistry & Molecular Genetics, University of Virginia Health System, Charlottesville, VA 22908-0733.

Angelman syndrome (AS) is a neurogenetic disorder characterized by severe mental retardation, 'puppet-like' ataxic gait with jerky arm movements, seizures, EEG abnormalities, hyperactivity and bouts of inappropriate laughter. Individuals with AS fail to inherit a normal maternal copy of chromosome 15q11-q13 usually (65-70% of all AS cases) as a result of de novo deletion of the maternal 15q11-q13 region. A small number (5-10%) of AS cases result from paternal uniparental disomy (UPD) for chromosome 15 or imprinting defects. Mutations of the gene encoding ubiquitin protein ligase E3A (UBE3A) are detected in AS cases not resulting from maternal deletion of 15q11-q13, paternal UPD or imprinting defect. UBE3A is transcribed predominantly from the maternal allele in brain but is expressed from both alleles in most other tissues. We have proposed that silencing of the paternal UBE3A allele is mediated by a brain-specific paternal noncoding antisense transcript (UBE3A-ATS). We show here that, in a mouse model of AS, maternal transmission of the *Ube3a* mutation leads to increased expression of the paternal *Ube3a-ATS*. Although it has been demonstrated in other cases of epigenetic regulation that antisense transcripts function in *cis* to silence target genes such as *Xist* and *Igf2r*, this is the first report to indicate that the putative target of antisense silencing can function to modulate the antisense transcript. Moreover, the observation that *Ube3a* modulates expression of *Ube3a-ATS* in *trans* is in contrast to the other examples of sense-antisense *cis* interactions. We are currently studying the potential mechanism associated with the *trans*-interaction and determining whether the expression of transcripts other than *Ube3a-ATS* are affected by mutation of *Ube3a*.

**Imprinting of the 5HT<sub>2A</sub> receptor in schizophrenia.** *M.S. Sodhi<sup>1,2</sup>, D. Ansari<sup>2</sup>, E. Sanders Bush<sup>1</sup>, P.J. Harrison<sup>2</sup>.* 1) Department of Pharmacology, Vanderbilt University, Nashville, TN, USA; 2) University Dept of Psychiatry, Warneford Hospital, Oxford UK.

The 5HT<sub>2A</sub> receptor (5HT<sub>2A</sub>R) is widely expressed in the human brain and evidence suggests that 5HT<sub>2A</sub>R may play a role in the aetiology and treatment of psychiatric disorders. Epigenetic factors such as imprinting, mRNA splicing and mRNA editing<sup>1</sup> can produce phenotypic variation, thus confounding the assumed relationship between genotype and phenotype. Imprinting of the 5HT<sub>2A</sub>R gene has been reported in 4 out of 18 individuals tested<sup>2</sup> and subsequently, partial imprinting of 5HT<sub>2A</sub>R has been detected in a larger sample<sup>3</sup>. Meta-analysis has demonstrated 5HT<sub>2A</sub>R allelic association with schizophrenia<sup>4,5</sup> but there are many studies contradicting these findings, which is common with association data. Therefore we have tested the hypothesis that imprinting of the 5HT<sub>2A</sub>R gene is polymorphic, and that this phenomenon explains the contradictory results of genetic association studies. The relative expression of the 5HT<sub>2A</sub>R T102 and C102 alleles was compared in post-mortem brain tissue in regions shown to be altered in schizophrenia, the dorsolateral prefrontal cortex (BA46) and hippocampus. 5HT<sub>2A</sub> T/C heterozygote individuals were identified by genotyping, revealing 9 schizophrenia cases and 8 controls, for which prefrontal cortex tissue was available and 6 cases and 6 controls with available hippocampal tissue. Results showed neither partial nor complete imprinting in any of the individuals tested. Moreover, no significant differences were detected between schizophrenia and control subjects in either BA46 (ANOVA F=0.44, ns.) or in hippocampus (ANOVA F=0.40, ns.) Therefore, imprinting of the 5HT<sub>2A</sub>R gene was not demonstrated in this study and if it does occur, it is probably too infrequent to alter the interpretation of genetic association data.

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Program Nr: 953 from 2003 ASHG Annual Meeting

**Family History of Diabetes Mellitus (DM) and Turner Syndrome (TS).** *V.K. Bakalov, M.M. Cooley, C.A. Bondy.*  
Developmental Endocrinology , NICHD-NIH, Bethesda, MD.

Previous studies reported an increased prevalence of DM in parents of individuals with X-chromosomal aneuploidies such as Turners and Klinefelters syndromes, leading to the hypothesis that abnormal glucose metabolism may increase the risk of offspring having sex chromosomal aneuploidy (Nature 221;175, 1969). We have revisited this question recently in 58 women with Turner Syndrome participating in an NIH clinical study. They were in good general health, without DM and highly functional with mean age of 39.1 years (range 25-59). Most were Caucasian, with 2 Hispanics and 1 Asian in the group. We found that 14/58 or ~24% of the women with TS had a parent with diabetes. We interviewed a control group of age- and race-matched normal women (n=82, 40.1 yrs) and found 21% had a parent with DM, not significantly different from the TS group. In both groups, the parent with DM was maternal ~ 50% of the time, not supportive of a paternal preponderance noted in previous studies. Finally, the theoretical chance of a 40 yr old in the U.S. having a parent with DM may be estimated based upon CDC data on the prevalence of DM in the 65-74 yr old population, which is 16% for Caucasian men and 12.5 for women. Assuming that mother and father get DM independently, the predicted chance that either parent has DM, using the CDC figures above, is 26.5%. Taken together, these observations tend to refute the notion that DM or impaired glucose metabolism contributes to generation of offspring with X-chromosomal aneuploidy.

**X;autosome balanced translocations and premature ovarian failure: chromatin organization around the breakpoints.** *F. Rizzolio<sup>1</sup>, F. Rossetti<sup>1</sup>, C. Sala<sup>1</sup>, S. Bione<sup>2</sup>, T. Pramparo<sup>3</sup>, E. Rossi<sup>3</sup>, M. Goegan<sup>1</sup>, R. Ricotti<sup>2</sup>, L. DalPra<sup>4</sup>, A. Marozzi<sup>5</sup>, W. Vegetti<sup>5</sup>, F. Torricelli<sup>6</sup>, O. Zuffardi<sup>3</sup>, D. Toniolo<sup>1,2</sup>.* 1) DIBIT, San Raffaele Scientific Inst., Milano, Italy; 2) IGM-CNR, Pavia, Italy; 3) Dept of Human Genetics, University of Pavia, Italy; 4) Dept of Human Genetics, University of Milano Bicocca, Milano, Italy; 5) Dept of Human Genetics, University of Milano, Milano, Italy; 6) Dept of Human Genetics, University of Firenze, Firenze, Italy.

Monosomies and rearrangements of the X chromosome are often associated to premature ovarian failure (POF), but no molecular explanation has been found for the observation. Several independent studies have mapped the breakpoints of X; autosomes balanced translocations associated with POF and have confirmed the critical region for POF between Xq21 and Xq26. We have analyzed 22 such translocations. Our study seems to exclude that rearrangements of the critical region are responsible for alterations in meiotic pairing as a small number of chromosomal rearrangements found in normal females have been mapped in close vicinity and in between rearrangements causing POF. They have however also failed to definitively identify genes for ovulation along the X. Only three genes have been found interrupted but for only one statistical evidence indicates that it may be a risk factor for POF. Moreover, the majority of the breakpoints was found in intergenic regions or in very gene poor regions. An alternative explanation for the phenomenon is a position effect of the breakpoint on gene(s), X linked or more likely autosomal, found in the vicinity of the breakpoints. To this aim we are mapping the autosomal breakpoints in some of the patients and we are studying the pattern of expression of the genes as well as the organization of the chromatin in the DNA surrounding the breakpoints. We have evidence that, in a number of the cases studied to date, the autosomal breakpoint is in gene rich regions and of the presence of characteristic chromatin organization in the region of the breakpoints.

**A new diagnostic method for functional chromosome abnormalities based on RNA-FISH.** *T. Kubota*<sup>1, 2</sup>, *M. Matsumura*<sup>2</sup>, *S. Morita*<sup>3</sup>, *I. Hatada*<sup>3</sup>, *K. Okumura*<sup>4</sup>, *T. Sado*<sup>5</sup>, *H. Sasaki*<sup>5</sup>, *Y. Goto*<sup>2</sup>. 1) Environment Health/Epigenetic, Faculty Med, Yamanashi Univ, Yamanashi, Japan; 2) Birth Def/Mental Retard, Natl Inst Neuroscience, NCNP, Tokyo, Japan; 3) Gene Research Center, Gunma Univ, Maebashi, Japan; 4) Life Science, Faculty of Bioresources, Mie Univ, Tsu, Japan; 5) Human Genet, Natl Inst Genet, Mishima, Japan.

Functional, not structural, abnormalities of chromosomes can cause genetic diseases. However, conventional cytogenetic assays cannot detect these abnormalities such as uniparental disomy in imprinted diseases, although molecular methods have been used in some diagnostic laboratories for this purpose. Therefore, we have developed a new cytogenetic method based on RNA-FISH, in which gene expression can be visualized on chromosomes. In this study, we prepared slides according to a standard interphase DNA-FISH protocol, in which cells were fixed in 3:1 methanol-acetic acid (Carnoy's fixative), except that solutions used were treated with an RNase inhibitor and denature steps were eliminated. Using a 1.6-kb *XIST* gene cDNA probe, we detected clear *XIST*-RNA signals on the nuclei of fresh human peripheral lymphocytes and EB virus-transformed lymphoblast cells. When RNA-FISH was performed followed by DNA-FISH with an X-centromere DNA probe, a single *XIST*-RNA signal was detected adjacent to one of two X-centromere DNA signals in female cells. These results were consistent with normal X-chromosome inactivation. Furthermore, we could detect distinct *XIST*-RNA signals in the peripheral blood samples that had been drawn 3 days earlier when these samples had been collected in the tubes containing a culture medium. These findings suggest that RNA-FISH can be performed in standard cytogenetic laboratories, and imply that it could be used as an alternative diagnostic method for diseases with functional chromosome abnormalities, such as imprinted diseases and diseases with a failure of X-chromosome inactivation.

**Epigenetic marks of tissue-specific and imprinted regulation of the human NDN gene.** *M.L. Hanel<sup>1</sup>, J.C.Y. Lau<sup>1</sup>, I. Paradis<sup>2</sup>, R. Drouin<sup>2</sup>, R. Wevrick<sup>1</sup>*. 1) Department of Medical Genetics, University of Alberta, Edmonton, Alberta, Canada; 2) Unité de Recherche en Génétique Humaine et Moléculaire, Centre de Recherche, Hôpital Saint-François d'Assise, CHUQ, Québec, Québec, Canada.

Allele-specific DNA methylation, histone acetylation and chromatin structure are recognized as epigenetic characteristics of imprinted genes and imprinting centers (ICs) and are also used to regulate tissue-specific gene expression of imprinted and non-imprinted genes. Overlapping signals modulating allele-specific and tissue specific expression exist at imprinted loci. We have now examined the epigenetic characteristics of NDN, a target gene of the cis-acting Prader-Willi syndrome IC, using sodium bisulfite sequencing to analyze DNA methylation and in vivo DNA footprinting to identify sites of DNA-protein interaction and altered chromatin configuration. We found that NDN lies in a domain of paternal allele-specific histone hyperacetylation and paternal allele-specific DNaseI sensitivity, in *necdin*-active cells. We observed a bias towards maternal allele-specific DNA hypermethylation of the promoter CpG island of NDN, independent of tissue-specific transcriptional activity. Sites of modified chromatin mark the parental alleles in expressing *necdin*, and in cells in which NDN is not expressed. Our results suggest that a layering of epigenetic information controls allele- and tissue-specific gene expression.

**MECP2 mutations in 217 patients with RTT syndrome.** *I. Kondo<sup>1</sup>, I. Ishihara<sup>2</sup>, M. Miyamoto<sup>1</sup>, T. Matsuishi<sup>3</sup>, Y. Yamashita<sup>3</sup>, K. Kuwajima<sup>4</sup>, T. Iwai<sup>5</sup>, S. Endo<sup>5</sup>, M. Sone<sup>5</sup>, H. Yamagata<sup>1</sup>, Y. Tabara<sup>1</sup>, I. Ohmori<sup>6</sup>, E. Oka<sup>6</sup>.* 1) Dept. of Med. Genet., Ehime Univ. Sch. of Med., Ehime, Japan; 2) Faculty of Nursing, Univ. of Occupat. and Env. Health, Japan, Fukuoka, Japan; 3) Dept. of Pediatr. and Child Health, Kurume Univ. Sch. of Med., Fukuoka, Japan; 4) Dept. of Pediatr., Ibaraki Pref. Handicapped Children's Hosp., Ibaraki, Japan; 5) Dept. of Pediatr., National Kagawa Children's Hosp. Kagawa, Japan; 6) Dept. of Child Neurol., Okayama Univ. Sch. of Med., Okayama, Japan.

Numerous mutations in the methyl-CpG-binding protein 2 gene (MECP2) have been reported in patients with Rett syndrome (RTT) and X-linked mental retardation (XLMR). A wide spectrum of phenotypic variability in patients with MECP2 mutation has been considered to be correlated with the pattern of X-inactivation and mutation type and location in the gene. However, some mutations reported in patients with RTT and XLMR might be rare DNA variations. To study phenotype-genotype correlations in MECP2 mutations in RTT, a total 217 patients with classic and atypical RTT were studied for mutations of the MECP2 by using DGGE and direct sequencing. Patterns of X-inactivation were determined using PCR analysis of the androgen receptor gene. A total 61 different mutations were identified in 137 (63.1%) patients with RTT. The most common mutations is T158M, identified in 21(15.3%) patients, following by R168X (11.1%), R270X(8.9%), R255X (8.1%) and R294X (7.4%) in 138 patients with classic RTT. Two missense mutations, R133C (8.8%) and R306C (5.2%), were also common in our study, but clinical phenotypes in these patients were atypical RTT including preserved speech variant and milder form of classic RTT. Almost all patients showed random X inactivation pattern, suggesting clinical phenotypes did not depend on skewed X inactivation. Three missense mutations, P176R, A201V, and G232A, were identified in patients with RTT, but these mutations were also detected in healthy Japanese, indicating non-pathogenic variations in Japanese. Then, rare polymorphic variations should be carefully distinguished for the detection of distinct DNA mutations responsible for clinical conditions.

**Beckwith-Wiedemann syndrome and isolated hemihyperplasia following ART: Association with paternal UPD of**

**11p15.** C. Clericuzio<sup>1</sup>, C. Shuman<sup>2,3,4</sup>, L. Steele<sup>3,5</sup>, J. Milisa<sup>1</sup>, P. Ray<sup>3,4,5</sup>, R. Weksberg<sup>2,3,4,6</sup>. 1) Ped-Div Dysmorph/Clinical Gen, Univ New Mexico Sch Medicine, Albuquerque, NM; 2) Div Clin & Metab Genetics, Hosp Sick Children, Toronto; 3) Dept Mol & Med Genetics, Univ Toronto, Toronto; 4) Research Inst, Hosp Sick Children, Toronto; 5) Dept Paed Lab Med, Hosp Sick Children, Toronto; 6) Dept of Paed, Hosp Sick Children, Toronto.

Beckwith-Wiedemann syndrome (BWS) is a prototypical imprinting disorder, caused largely by epigenetic alterations of imprinted genes in the chromosomal region 11p15.5. Fifteen infants with BWS have been reported following conceptions with assisted reproductive technology (ART). Molecular genetic analysis of 7 of these cases has identified imprinting defects involving hypomethylation of KvDMR1. These data taken together with a report of 3 cases of ART-associated Angelman syndrome due to imprinting defects supports an association between ART and imprinting disorders. Here, we report 1 child with BWS and another with isolated hemihyperplasia (HH) who were conceived by ART (IVF) and who were found to have uniparental disomy (UPD) of chromosome 11p15.

Pt 1: A 36 week gestation male twin was diagnosed with BWS and later developed a Wilms tumor. Genetic studies on foreskin fibroblasts from the patient and parental blood DNA showed somatic mosaicism for paternal UPD of 11p15. Pt 2: A term female twin was diagnosed with right HH at 6 mo, following presentation with Stage 3 hepatoblastoma. Genetic studies of the patient's blood DNA revealed likely paternal UPD 11p15; parental samples have been requested for confirmation.

The finding of UPD 11p15 in these 2 cases suggests that errors in post-zygotic recombination that lead to UPD may be associated with ART. The development of tumors in these cases is not surprising, as BWS with UPD 11p15 is known to have a significant cancer risk. Moreover, the clinical spectrum associated with UPD 11p15 is now believed to encompass HH in addition to BWS. Hence, ART may be associated not only with epigenetic imprinting errors, but may also influence the risk of somatic mitotic recombination.

**Identification of acquired somatic mutations in the chromatin remodelling factor ATRX in the thalassemia myelodysplasia syndrome (ATMDS).** *R.J. Gibbons<sup>1</sup>, A. Pellagatti<sup>2</sup>, D. Garrick<sup>1</sup>, W.G. Wood<sup>1</sup>, N. Malik<sup>1</sup>, H. Ayyub<sup>1</sup>, C. Langford<sup>3</sup>, J. Boulwood<sup>2</sup>, J.S. Wainscoat<sup>2</sup>, D.R. Higgs<sup>1</sup>.* 1) MRC Molecular Haematology Unit, WIMM, John Radcliffe Hospital, Oxford, UK; 2) LRF Molecular Haematology Unit, NDCLS, John Radcliffe Hospital, Oxford, UK; 3) Sanger Centre, Hinxton, Cambridge, UK.

Thalassemia myelodysplasia syndrome (ATMDS) is a rare hematological disorder of clonal origin, associated with hypochromic microcytic anemia. Myeloid rather than lymphoid progenitors are affected. Therefore to obtain a population of cells affected by an acquired mutation in ATMDS, purified (>95%) granulocytes were isolated from the peripheral blood of a newly diagnosed ATMDS patient. Using microarray analysis to identify genes whose expression might be perturbed, ATRX expression was found to be reduced to 3% of normal. Sequence analysis of the ATRX gene revealed a G>A mutation in the canonical splice donor site (GT) of intron 1. This mutation, though present in granulocytes, was absent from buccal cells and a lymphoblastoid cell line from this patient. Using archival bone marrow samples, the ATRX gene was sequenced from cDNA in other ATMDS cases. A C>G (S79X) mutation in exon 4 was identified in one other patient. A mixture of mutant and wildtype sequence was detected in DNA from bone marrow and peripheral blood and this was confirmed by subcloning the two species.

Whereas the hematological phenotype in patients with inherited mutations affecting this gene (ATR-X syndrome) is subtle, in ATMDS the anemia is profound and is associated with the virtual absence of globin expression. To date, none of the constitutive ATRX mutations appears to be null. It seems likely, however, that mutations seen in ATMDS cases are true nulls and represent a tissue specific knockout. These findings cast new light on this pleiotropic co-factor which appears to be essential rather than merely a facilitator of globin gene expression.

**The parental origin of the normal X chromosome affects blood pressure in Turner Syndrome.** *C.A. Bondy<sup>1</sup>, M. Cooley<sup>1</sup>, V.K. Bakalov<sup>1</sup>, A.R. Zinn<sup>2</sup>.* 1) Developmental Endocrinology, National Institute of Child Health, NIH, Bethesda, MD; 2) McDermott Center and Dept. of Internal Medicine Univ. Texas Southwestern Medical School, Dallas, TX.

Turner syndrome (TS) arises from partial or complete X monosomy and is characterized by short stature, ovarian failure, distinct neurobehavioral traits, and cardiovascular problems including essential hypertension. Skuse et al. (1997) reported that individuals retaining the paternal X chromosome (Xp) had better psychosocial functioning than individuals whose normal X chromosome was maternal (Xm), suggesting that a gene or genes situated on the X-chromosome and involved in brain development might be subject to genomic imprinting. In the present study we investigated the possibility that the expression of certain cardiovascular features of TS might be dependent upon the parental source of the normal X. Thus we performed 24-hr ambulatory blood pressure (BP) monitoring on women with TS aged 18-42 participating in an NIH study of Turner syndrome. Women with coarctation of the aorta or any other known cause of hypertension, and those taking blood pressure lowering drugs were excluded. Genetic material was obtained from study subjects and parents under an IRB-approved protocol, and all participants signed informed consents. Conclusive data concerning parental origin of the single normal X-chromosome was obtained for 31 subjects; of these, 23 were maternal and 8 paternal in origin. Ages and body mass index were similar in the two groups. However, the mean arterial blood pressure (MABP) was significantly higher in the Xm group: Xm 892 vs. Xp 792 mm Hg ( $P=0.007$ ). An ANCOVA analysis assessing the impact of age and body mass in addition to X origin on MABP variation confirmed statistical significance for the elevation of blood pressure related to Xm ( $P=0.04$ ). These observations suggest that there is genomic imprinting of X-chromosome genes involved in BP regulation, with moderating effects active from paternal allele(s) or, alternatively, hypertensive effects from maternal allele(s).

**Identification and Functional Analysis of Human LIT1 (KCNQ1OT1) Promoter.** *M. Du<sup>1</sup>, W. Zhou<sup>1</sup>, L. Beatty<sup>1</sup>, R. Weksberg<sup>2</sup>, P. Sadowski<sup>1</sup>.* 1) Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, CANADA; 2) Division of Clinical and Metabolic Genetics, Hospital for sick Children, Toronto.

KvDMR is a differentially methylated region (DMR) in intron 10 of the KCNQ1 gene. KvDMR acts as an imprinting control center that regulates the expression of several adjacent imprinted genes in cis on human chromosome 11p15.5. It is also thought to contain the promoter for the LIT1 (KCNQ1OT1) transcript, a long, maternally imprinted, antisense transcript of the KCNQ1 gene. Loss of imprinting of LIT1 and loss of methylation of the KvDMR are seen in about 50% of patients with Beckwith-Wiedemann syndrome. Our previous investigations showed that the KvDMR also possesses strong silencer activity, thereby supporting a possible regulatory function in control of the imprinting gene cluster. In the present study, we have identified and functionally analyzed the human LIT1 promoter. We tested a series of DNA fragments from the KvDMR using a luciferase reporter assay in HEK293, HepG2 and Hela cells. A 581bp fragment exhibits very strong promoter activity while larger fragments with additional upstream and/or downstream sequences show reduced activities, suggesting possible upstream or downstream inhibitory elements. Interestingly, promoter activity has been detected in the reverse orientation from a tested subfragment of the 581bp putative promoter, indicating the possibility of sense transcripts originating in intron 10 of the KCNQ1 gene. The putative LIT1 promoter lacks a TATA-box but contains 4 CAAT-box and 4 SP1 sites as well as other potential binding sites for transcription factors. When the putative promoter fragments were methylated in vitro before transfection, the luciferase activities in the transfectants were markedly reduced. This observation supports the hypothesis that the imprinting of the LIT1 transcript is due to methylation in the maternal LIT1 promoter. We are determining the initiation site of the LIT1 transcript and the CpG residues in the minimal promoter fragment that are critical for LIT1 promoter activity. Our study suggests that functional analysis of imprinted promoters will provide insight into the mechanisms of genomic imprinting and related diseases.

**Heritability and identification of quantitative traits loci in eye morphometry: evidence of imprinting.** *G. Biino<sup>1</sup>, M.A. Palmas<sup>2</sup>, C. Corona<sup>2</sup>, R. Sulis<sup>2</sup>, I. Zucca<sup>3</sup>, M. Fossarello<sup>3</sup>, A. Serra<sup>3</sup>, M. Pirastu<sup>1</sup>*. 1) Institute of Population Genetics, CNR - Shardna Life Sciences, Alghero, Italy; 2) Shardna Life Sciences, Cagliari, Italy; 3) Eye Clinic, University of Cagliari, Cagliari, Italy.

Our aim is to assess the heritability of biometric ocular traits and to perform a genome wide scan to identify the associated QTLs. This study was conducted in Talana, an isolated village in eastern Sardinia whose demographic history exhibits ancient origin, low number of founders, high inbreeding and genealogical trees reconstructable from 1640. Volunteers from Talana (N=789, aged 5-89 years) have undergone a complete eye examination including ocular biometry: axial length (AL), anterior chamber depth (ACD) and corneal curvature (CC) were measured. Heritability analysis was performed by means of two methods: parent-offspring regression models on 201 nuclear families and variance component models on 3-4 generations pedigrees. Two and multipoint linkage analysis were performed using 654 microsatellites. The heritability estimates obtained by means of parent offspring regressions and variance components were consistent and significant ( $p < 0.05$ ). CC (mean value 7.67 0.25) had an heritability estimate of 62%. AL (mean value 23.49 0.91) and ACD (mean value 3.45 0.34) were found to have significantly different variances ( $P < 0.01$ ) in males and females so that heritability had to be calculated separately for each sex in the parent offspring regression models. AL had an estimated heritability in females of 31% and in males of 60%, whereas ACD had an estimated heritability in females of 47% and of 44% in males. Suggestive evidences of linkage in multipoint analysis were identified on chromosomes 6, 7, 14 and 15 for ACD, on chromosomes 6 and 12 for CC and on chromosome 2 for AL. To further examine the observed differences in parental transmission we performed Haseman Elston regressions, extended to accommodate parent of origin effects, on a larger sample of 231 nuclear families for markers with evidence of linkage. Preliminary results for AL suggest a preferentially paternal expression. Subsequent findings on all morphometric traits will be discussed.

**A randomized therapeutic trial in Angelman syndrome using betaine and folic acid.** C.A. Bacino, S. Peters, J. Goddard-Finegold, O. Smith, W. O'Brien, N. Madduri, D. Glaze, L. Shinawi, A.L. Beaudet. Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Angelman syndrome (AS) is caused by maternal deficiency of UBE3A which encodes E6-AP ubiquitin-protein ligase. UBE3A is subject to genomic imprinting, and expression from the maternal chromosome is essential to prevent AS. The report of MTHFR deficiency in a patient with a typical AS phenotype in the absence of a known molecular defect causing AS raised the possibility that decreased folate effect might silence the maternal allele for UBE3A. This led to the reciprocal hypothesis that high folate effect might promote leaky expression of the paternal allele for UBE3A providing a therapeutic benefit in AS. A clinical trial was developed to test if high intake of folic acid and betaine might provide clinical benefit in AS. Twenty patients with AS were enrolled in the study for 1 year. The subjects were randomized with half receiving drugs and the other half placebo. A nutritional evaluation and hematologic and biochemical studies were performed. The study included developmental evaluations performed by the Bayley Scales of Infant Development and by the Preschool Language Scale-3 to assess expressive and receptive language skills. In addition the Vineland Adaptive Behavior Scales, Interview Edition was done to assess parent reports of adaptive behavior. Of the 20 children enrolled in the study, 8 met criteria for the diagnosis of autism according to the ADI-R and the ADOS-G. When medication and placebo groups were compared, there were no significant differences between the two groups. When the autistic and nonautistic patients were analyzed separately, there was a trend of improvement in some developmental parameters in the nonautistic medication group for the Preschool Language Scales Composite scores as well as Expressive Communication scores. Although not statistically significant, Bayley Mental Scale scores were higher in the nonautistic medication group compared to the nonautistic placebo group. This may indicate a beneficial effect of folic acid and betaine in a subset AS children. Further studies are underway to increase sample size and assess whether this trend persists.

**The use of Me-RDA to identify novel DMRs on mouse Chr 6 and 11.** *D. Monk*<sup>1</sup>, *L. Bentley*<sup>1</sup>, *C. Beechey*<sup>2</sup>, *R. Smith*<sup>3</sup>, *P. Arnaud*<sup>3</sup>, *J. Peters*<sup>2</sup>, *M. Preece*<sup>4</sup>, *P. Stanier*<sup>1</sup>, *G. Kelsey*<sup>3</sup>, *G. Moore*<sup>1</sup>. 1) IRDB, Imperial College, London, UK; 2) MRC Mammalian Genetics Unit, Harwell, UK; 3) Developmental Genetics Programme, The Babraham Institute, Cambridge, UK; 4) Institute of Child Health, UCL, London, UK.

Silver-Russell syndrome is characterised by pre- and postnatal growth restriction. Maternal uniparental disomy for Chr 7 (mUPD7) has been demonstrated in ~10% of cases, suggesting a role for genomic imprinting in its causation. Two candidate gene regions have been mapped on human Chr 7. The first, 7p11.2-p13 encompass the imprinted *GRB10* gene, whereas the second maps to 7q31-qter. This second region contains the imprinted gene cluster of *MEST*, *MEST1T1*, *CIT1/COPG2IT1* and *CPA4*. No coding or epigenetic mutations have been found for these genes in any SRS patients to date, suggesting that there are other imprinted genes which map to human Chr 7.

Approximately 50% of human Chr 7 maps to regions of the mouse genome that presents with imprinted phenotypes when uniparentally inherited. Interestingly both candidate regions on human Chr 7 map to imprinted regions in the mouse that show growth restriction when maternally duplicated (MatDp). Proximal mouse Chr 11 is orthologous to 7p11.2-p13, whereas the proximal mouse Chr 6 is orthologous to 7q32-qter.

In order to identify novel imprinted candidate genes responsible for the growth phenotypes, we have screened for unmethylated fragments from differentially methylated regions (DMRs) that are associated with imprinted genes. Methylation-sensitive representational difference analysis (Me-RDA) was performed on mice with uniparental duplication proximal to the T40(11:7)Ad breakpoint on mouse Chr11 and separately on distal 6 to the T(4:6)77H breakpoint on mouse Chr 6. Preliminary analysis has demonstrated recovery of *U2af-rs1* and *Grb10* confirming the successful application of the technique. We report here the methylation status of candidate loci isolated from these two screens.

**Investigation of parent of origin effects for Autism susceptibility loci on chromosomes 2, 7 and 15.** *J. Jaworski<sup>1</sup>, A.E. Ashley-Koch<sup>1</sup>, M. Menold<sup>1</sup>, R.K. Abramson<sup>2</sup>, G.R. DeLong<sup>1</sup>, H.H. Wright<sup>2</sup>, M.L. Cuccaro<sup>1</sup>, J.R. Gilbert<sup>1</sup>, M.A. Pericak-Vance<sup>1</sup>.* 1) Duke University Medical Center, Durham, NC; 2) University of South Carolina, Columbia, SC.

Genomic screen and cytogenetic data implicate loci on chromosomes (Ch) 2, 7, and 15 in susceptibility for autistic disorder (AD). We have previously shown the GABRB3 locus on Ch 15q11-q13 to be linked to AD in a subset of 23 families with a high degree of insistence on sameness as identified from Ordered Subset Analysis (OSA). To determine whether these loci exhibit signs of parent of origin effects, we examined the 23 OSA and an additional 187 multiplex AD families. Using ASPEX to examine parental-specific IBD sharing in affected siblings, we saw excess paternal sharing for three of our peak markers on Ch 2: D2S1776 ( $p=0.03$ ), D2S2261 ( $p=0.02$ ), and D2S425 ( $p=0.04$ ). Excess paternal sharing was also observed on Ch 7, with D7S2527 ( $p=0.02$ ), D7S496 ( $p=0.05$ ), D7S495 ( $p=0.03$ ), and D7S640 ( $p=0.02$ ), as we had previously reported. On Ch 15, in the OSA families, GABRB3 SNPs in intron 6 ( $p=0.03$ ) and the promoter region ( $p=0.01$ ) showed evidence for excess maternal sharing. GENEHUNTER-IMPRINTING was used to maximize the heterogeneity LOD score (hetlod) over five heterozygote penetrance models; dominant, co-dominant, recessive, paternal imprinting and maternal imprinting. For Ch 2, D2S1776 had a peak 2pt hetlod of 1.8, maximizing under the maternal imprinting model. For Ch 7, the peak marker was D7S496, which maximized under the dominant model (hetlod=1.12). For Ch 15, in the OSA subset, the maximum hetlod was observed with the GABRB3 promoter SNP, under the paternal imprinting model (hetlod=1.65). These results are intriguing, but do not provide definitive evidence for parent of origin effects in AD. Due to the complex mode of inheritance, it is likely that a much larger sample size will be needed for more conclusive evidence to emerge.

**Isolation of a mouse snoRNA host gene from the *Snurf-Snrpn* locus and association with imprint establishment.**

*K. Mapendano*<sup>1</sup>, *T. Kishino*<sup>1</sup>, *N. Niikawa*<sup>1</sup>, *R.D. Nicholls*<sup>2</sup>, *T. Ohta*<sup>1</sup>. 1) Department of Human Genetics, Nagasaki University, Nagasaki, Japan; 2) Department of Psychiatry, University of Pennsylvania, Philadelphia, PA.

Prader-Willi (PWS) and Angelman syndromes are neurobehavioral disorders caused by loss of function of paternally or maternally expressed genes, respectively, at 15q11-q13. Five families of C/D box snoRNAs have been mapped between *SNURF-SNRPN* and *UBE3A* in this chromosome region. These snoRNAs are expressed predominantly from the brain and only from the paternal allele. Previous studies in mouse and human suggested that the snoRNA genes may be involved in the PWS phenotype. The human snoRNA host gene was previously identified as a large transcript extending from *SNRPN* 3'-alternatively spliced exons into *UBE3A*. This transcript was assumed to start from the *SNURF-SNRPN* promoter and has been proposed to control *UBE3A* imprinting in *cis* as an antisense transcript, although the latter may represent extended transcripts in the absence of paternal *UBE3A* transcription due to a *trans*-mechanism. To elucidate the mechanism associated with snoRNA expression, we have isolated the mouse snoRNAs and the host gene. Only four of the five human classes of snoRNA were found conserved in mouse. We then performed 5'- or 3'-RACE and nested RT-PCR with mouse *IC*, *Snurf-Snrpn* and *Ipw* primers. 5' RACE and nested RT-PCR showed that the mouse *IC* transcripts, *Snurf-Snrpn* and *Ipw* are continuous transcripts. RT-PCR and northern analyses using multiple tissues showed that these *IC-IPw* transcripts and the snoRNAs are expressed in mouse brain and ovary. High expression of the snoRNA host gene from the ovary suggests that the snoRNAs may be required in oocyte function. Further, the germline-specific snoRNA host gene expression suggests this derives from the 5' *IC* promoters and not the 3' *Snurf-Snrpn* promoter, based on a differential expression of these promoters in female and male germ cells. The different chromatin structure from the *IC* to *Ube3a* in female and male germlines as a consequence of the differential expression of the *IC-Snurf-Snrpn*-snoRNA host gene may play a role in establishment of the parental imprint in this region.

**The methylation status of imprinted genes in murine and human MTHFR deficiency.** *M. Shinawi, V.R. Sutton, A. Beaudet.* Molecular Human Genetics T617 , Baylor College of Medicine , Houston , TX.

MTHFR plays an important role in folate metabolism by catalyzing the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Methionine is the immediate precursor of S-adenosyl-L-methionine, the major intracellular methyl donor for methylation reactions. Recently, it has been shown that *Mthfr* deficient mice have DNA hypomethylation and the C677T mutation in human *MTHFR* affects genomic DNA methylation. We hypothesized that MTHFR may be required for the regulation of differentially methylated regions (DMRs) of imprinted genes and a deficiency may disrupt this mechanism. We checked the differential methylation of 4 different imprinted genes (*Snrpn*, *Necdin*, *H19*, and *Rasgrf1*) in different tissues of the *Mthfr*<sup>-/-</sup> mice. We also examined the methylation of *SNRPN* and *UBE3A* in the lymphoblasts and fibroblasts from 2 children with MTHFR deficiency who have presented with severe psychomotor retardation and dysmorphic features. Furthermore, the DMR of *Snrpn* in *Mthfr*<sup>-/-</sup> mice was sequenced after bisulfite treatment to precisely evaluate the methylation of the different CpGs at this region compared to *Mthfr*<sup>+/-</sup> and wild type mice. The methylation pattern of the DMRs was examined by Southern blot analysis after double digestion with methylation sensitive enzymes, and no change was demonstrated in the methylation pattern of these imprinted genes in the different tissues of the *Mthfr*<sup>-/-</sup> mice compared to wild type and *Mthfr*<sup>+/-</sup> mice. Bisulfite sequencing of the *Snrpn* DMR didn't show hypomethylation in the homozygous and heterozygous *Mthfr* deficient mice. Treating fibroblast cell lines from normal controls with different concentrations of folate did not change the methylation status of the 3'-DMR of *UBE3A*, but results from MTHFR deficient patients and cell lines treated with other metabolites and drugs are still pending. In conclusion, our results do not support an essential role for MTHFR in the methylation of the regulatory elements of imprinted genes in mice and human. These results may also suggest an alternative pathway for methylation of these regions or their sparing from the general hypomethylation that was observed in MTHFR-deficient humans and mice.

**Identification and analysis of *cis*-acting elements associated with the PWS-IC.** *S. Rodriguez-Jato*<sup>1</sup>, *D.J. Driscoll*<sup>1</sup>, *R.D. Nicholls*<sup>2</sup>, *T.P. Yang*<sup>1</sup>. 1) Univ of Florida College of Medicine, Gainesville, FL; 2) Univ of Pennsylvania, Philadelphia, PA.

The Prader-Willi and Angelman syndrome (PWS/AS) region includes a cluster of imprinted genes that are coordinately regulated by an imprinting center (IC) that spans the 5' region of the *SNURF-SNRPN* locus. The IC has a bipartite structure where the PWS-IC is postulated to maintain the paternal imprint in early embryogenesis, while the AS-IC is responsible for maternal imprint establishment. The *SNURF-SNRPN* promoter is implicated in PWS-IC function, while both an uncharacterized genetic element and alternative upstream *SNURF-SNRPN* promoters have been implicated in the function of the AS-IC. The PWS-IC contains major nuclease hypersensitive sites (HS) associated with the *SNURF-SNRPN* promoter region and 1st intron (adjacent to the IC), both specific to the paternal allele. *In vivo* footprint analysis of the promoter region has identified multiple factor binding sites, 4 specific to the paternal allele and 1 specific to the maternal allele. The paternal sites have been shown previously by *in vitro* studies to bind the NRF-1 and CTCF transcription factors. We have now found that some, but not all, of these sites affect promoter function in transient expression assays. Further, we have identified a novel position-dependent and orientation-independent activator associated with the intronic HS. This enhancer element preferentially activates the *SNURF-SNRPN* promoter relative to the *UBE3A* or *MKRN3* promoters. It appears to be a complex regulatory unit from which we have mapped a subregion that sustains high levels of *SNURF-SNRPN* promoter activity. Sequence comparison of this intronic activator with the homologous region in mouse identified several highly conserved sites within the subregion, including potential binding sites for NRF-1, and for YY1, a transcription factor implicated in imprinted expression of the *Peg3* gene. We are investigating the effect of this intronic activator on upstream *SNURF-SNRPN* promoters. This activator, together with *cis*-acting elements of the *SNURF-SNRPN* 5' region and upstream promoters, respectively, may play a role in the function of the PWS-IC and the AS-IC.

**An Approach to the Epigenetics of a Complex Trait.** *H.T. Bjornsson*<sup>1,2</sup>, *A. Aina*<sup>3</sup>, *F. Witter*<sup>3</sup>, *A.P. Feinberg*<sup>1,2</sup>. 1) Program in Human Genetics, Johns Hopkins University, Baltimore, MD; 2) Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 3) Department of Obstetrics and Gynecology, Johns Hopkins University School of Medicine.

Deciphering the genetic contribution of complex traits has turned out to be more of a challenge than previously thought. This might be because the epigenetic contribution has widely been disregarded in studies. We have selected birth weight as a well-defined candidate complex trait to define how epigenetics may contribute to complex traits. Birth weight was chosen because studies in mice show that imprinting plays a significant role in birth weight determination, and several human types of uniparental disomy suggest the same is true for humans. Indeed, the Haig Hypothesis states that genomic imprinting arose as an evolutionary mechanism to deal with the natural conflict between maternal and paternal genomes in prenatal growth. It predicts that birth weight is determined in part by the expression of imprinted genes.

As a first test of this idea, we have developed quantitative allele-specific expression ratio assays for a panel of imprinted genes including IGF2, MEG3, PEG3, and PLAGL1. We are analyzing placental, white cell and umbilical samples from human newborns, as well as neonatal weight, gestational age, and relevant maternal factors such as gestational diabetes. At this writing (06/03), we do not have data on the relationship between birth weight and allele-specific expression. A greater understanding of human birth weight determination may as well be important because of the link between several adult onset diseases and low birth weight.

**Evaluation of *MLH1* gene expression following reactivating treatments in colorectal cancer cell lines with hypermethylated *MLH1* promoter.** *M. Genuardi*<sup>1</sup>, *I. Zito*<sup>2</sup>, *E. Lucci-Cordisco*<sup>2</sup>, *L. Boccuto*<sup>2</sup>, *G. Chichierchia*<sup>2</sup>, *G. Neri*<sup>2</sup>. 1) Med Gen, Clin Pathophysiology, Univ Florence, Florence, Italy; 2) Medical Genetics, Catholic University School of Medicine, Rome, Italy.

Promoter hypermethylation is one of the leading mechanisms accounting for gene inactivation in human cancer. This holds true also for the human mismatch repair gene *MLH1*, which shows promoter hypemethylation in about 10-15% of sporadic colorectal cancers (CRCs). Our purpose was to establish the level of *MLH1* reactivation in HCA7 and RKO cell lines (both MMR-deficient due to *MLH1* promoter hypermethylation), following treatments with the demethylating agent 5-azadC alone and in combination with the histone deacetylase inhibitors (HDACIs) Na-butyrate (BA) and 4-phenyl-butyrate (PBA), and the probable histone acetylator acetyl-L-carnitine (ALC). By means of Real Time-PCR and Western Blotting we observed that 0.1 and 0.2 M 5-azadC resulted in reactivation of *MLH1* transcription within 3 days in HCA7 and RKO cells, respectively. Expression levels increased with increasing concentrations of the substance and with time of exposure and persisted for at least 2 weeks following treatment suspension. Combined 5-azadC/BA treatment resulted in a synergistic effect on *MLH1* expression only with low BA doses, whilst preliminary results with 5-azadC/PBA showed a synergistic effect with a maximum of *MLH1* expression after 16 h. The 5-azadC/ALC experiments gave little or no extra reactivation as compared to treatment with 5-azadC only. These data confirm that *MLH1* silencing can be overcome following treatments with demethylating agents and enhanced following combined treatments with HDACIs. They also show that the magnitude of this effect depends on dosage of each agent, especially for combined treatments, on time of exposure, and on the type of cell line. Such reactivated cells may now respond to drugs that are normally tolerated by MMR-deficient cell lines (e.g. alkylating agents). This work was supported by AIRC and MIUR grants to G. N. and M.G., respectively.

**Regulation of neural gene expression in the CNS by DNA methylation.** *S. Fouse, G. Fan.* Human Genetics, UCLA School of Medicine, Los Angeles, CA.

DNA cytosine methylation plays an important role in regulating gene expression during mammalian development. CpG methylation in the promoter region leads to the suppression of gene transcription whereas unmethylated promoter is usually correlated with open chromatin structure allowing for active gene transcription. We have previously generated a line of transgenic mice that exhibit significant DNA demethylation in the entire central nervous system (CNS) due to conditional deletion of the maintenance DNA methyltransferase I gene (*J. Neurosci.* 21, 788, 2001). To determine whether a reduction in DNA methylation affects gene expression in the CNS, we used DNA microarrays to compare gene expression profiles between control and demethylated CNS samples. Significant changes of mRNA levels were observed in approximately 45 genes (0.71%) in the mutant CNS at the embryonic (E) day 18. Among the known genes are signaling molecules and enzymes, cell death-related genes, and synaptic proteins. Surprisingly, ten up-regulated genes belong to class I MHC molecules, which are normally detected at very low levels in the CNS compared to other somatic tissues. We have confirmed the array results with Northern blots and RT-PCR analysis. Our results indicated that DNA methylation does suppress expression of a subset of genes in the CNS during development. We are currently addressing the molecular mechanism by which DNA methylation inhibits expression of MHC I molecules and other neural genes in the CNS.

**SLIT2 promoter methylation analysis in neuroblastoma, Wilms' tumours and renal cell carcinoma.** *D. Astuti<sup>1</sup>, N.F. da Silva<sup>2</sup>, A. Dallol<sup>1</sup>, D. Gentle<sup>2</sup>, T. Martinsson<sup>3</sup>, P. Kogner<sup>4</sup>, R. Grundy<sup>1, 5</sup>, T. Kishida<sup>6</sup>, M. Yao<sup>6</sup>, F. Latif<sup>1, 2</sup>, E.R. Maher<sup>1, 2</sup>.* 1) Section of Medical and Molecular Genetics, Department of Paediatrics and Child Health, University of Birmingham, The Medical School, Edgbaston, Birmingham, B15 2TT, UK; 2) Cancer Research UK Renal Molecular Oncology Research Group, University of Birmingham, The Medical School, Edgbaston, Birmingham, B15 2TT, UK; 3) Department of Clinical Genetics, Gothenburg University, Sahlgrenska University Hospital/Ostra, S-416 85 Gothenburg, Sweden; 4) Childhood Cancer Research Unit, Department of Women and Child Health, Karolinska Institute, Karolinska Hospital, S-171 76 Stockholm, Sweden; 5) Department of Paediatric Oncology, Birmingham Childrens Hospital, UK; 6) Yokohama City University School of Medicine, Yokohama, Japan.

The 3p21.3 RASSF1A tumour suppressor gene (TSG) provides a paradigm for TSGs inactivated by promoter methylation rather than somatic mutations. Recently we identified frequent promoter methylation without somatic mutations of SLIT2 in lung and breast cancers. These findings prompted us to investigate SLIT2 methylation in these three human cancers. We analysed 49 neuroblastoma (NB), 37 Wilms tumours and 48 RCC and detected SLIT2 promoter methylation in 29% of neuroblastoma, 38% of Wilms tumours and 25% of RCC. Previously we had demonstrated frequent RASSF1A methylation in the same tumour series and frequent CASP8 methylation in the neuroblastoma and Wilms tumour samples. However there was no significant association between SLIT2 promoter methylation and RASSF1A or CASP8 methylation in neuroblastoma and RCC. In Wilms tumour there was a trend for a negative association between RASSF1A and SLIT2 methylation although this did not reach statistical significance. No associations were detected between SLIT2 promoter methylation and specific clinicopathological features in the tumours analysed. These findings implicate SLIT2 promoter methylation in the pathogenesis of both paediatric and adult cancers and suggest that further investigations of SLIT2 in other tumour types should be pursued.

**Epigenetic inactivation of SLIT genes in human cancers and in sputum from lung cancer patients and ductal carcinoma in situ from breast cancer patients.** *R.E. DICKINSON, A. DALLOL, E.R. MAHER, F. LATIF.* Department of Paediatrics and child health, Section of Medical and Molecular Genetics, University of Birmingham, Birmingham, MIDLANDS, U.K.

In *Drosophila* the Slit gene product, a secreted glycoprotein acts as a midline repellent to guide axonal development during embryogenesis. Three human Slit gene orthologues have been characterized and recently we reported frequent promoter region hypermethylation and transcriptional silencing of SLIT2 in lung, breast, colorectal and glioma cell lines and primary tumors. Furthermore, re-expression of SLIT2 inhibited the growth of cancer cell lines so that SLIT2 appears to function as a novel tumor suppressor gene. We analyzed the 5' CpG island of the SLIT3 gene in tumor cell lines and primary tumors for hypermethylation. SLIT3 was found to be methylated in breast, lung, colorectal and in glioma tumor cell lines. Hypermethylation of SLIT3 in tumor cell lines correlated with loss of gene expression and treatment with a demethylating agent reactivated SLIT3 gene expression. In primary breast tumors SLIT3 was methylated in 16% of primary breast tumors, 35% of gliomas and in 38% of colorectal tumors. Direct sequencing of bisulfite modified DNA from methylated tumor cell lines and primary tumors demonstrated that majority of the CpG sites analyzed were heavily methylated. To determine the extent of SLIT2 promoter hypermethylation in early breast tumorigenesis, we analyzed ductal breast carcinoma and corresponding ductal carcinoma in situ (DCIS) from breast cancer patients and DCIS without invasive cancer. 8/12 (67%) tumors were methylated, there was 100% concordance between tumor and corresponding DCIS methylation. Furthermore 3 of 10 DCIS without invasive cancer also underwent SLIT2 methylation. We also analyzed sputum and bronchial lavage samples from lung cancer patients for SLIT2 promoter region hypermethylation. SLIT2 methylation was demonstrated in 67% of exfoliative material from lung cancer patients. These findings further implicate the slit-roundabout-semaphorin gene family in tumor development and suggests a novel, and common underlying theme for these molecules in tumor suppression.

**DNA methylation inhibits STAT signaling and prevents precocious astrogliogenesis during CNS development.** *G. Fan*<sup>1</sup>, *K. Martinowich*<sup>2</sup>, *M. Chin*<sup>1</sup>, *F. He*<sup>2</sup>, *S. Fouse*<sup>1</sup>, *K. Shuai*<sup>3</sup>, *Y.E. Sun*<sup>2</sup>. 1) Human Genetics, UCLA School of Medicine, Los Angeles, CA; 2) Psychiatry and Pharmacology, UCLA School of Medicine, Los Angeles, CA; 3) Medicine and Biological Chemistry, UCLA School of Medicine, Los Angeles, CA.

DNA methylation has been implicated in regulating gene transcription, genomic imprinting, and X-inactivation in mammalian development. We report here that DNA methylation plays a critical role in controlling the timing of glial cell differentiation in the central nervous system (CNS). A number of astroglial differentiation-related genes are methylated and silenced during the neurogenic period in the mouse CNS but become demethylated and activated when CNS progenitors gain competence for gliogenesis. To examine if such a demethylation is causally linked to the initiation of gliogenesis, we used the cre/loxP system to achieve conditional gene deletion of DNA methyltransferase I (Dnmt1) and accelerated demethylation in CNS neural progenitor cells (NPC) (Fan, et al. *J. Neurosci.* 21, 788, 2001). DNA hypomethylation in NPCs enhanced activation of the astroglial JAK-STAT pathway and caused precocious astroglial differentiation throughout the embryonic CNS in vivo and in vitro. Demethylation in the promoters of the STAT1 gene and astroglial marker genes is accompanied by the dissociation of transcription repressor proteins such as methyl-CpG binding protein MeCP2 and a shift of the chromatin structure from a silent state into a permissive configuration for active gene transcription. Our data demonstrates that DNA methylation is an important mechanism to control the activation of JAK/STAT signals and to regulate the onset of cell-type specification during mammalian CNS development.

**De novo DNA methylation in neuronal and glial differentiation.** *M.H. Chin<sup>1</sup>, H. Wu<sup>2</sup>, S. Yuan<sup>2</sup>, E. Li<sup>3</sup>, Y.E. Sun<sup>2</sup>, G. Fan<sup>1</sup>.* 1) Human Genetics, UCLA School of Medicine, Los Angeles, CA; 2) Psychiatry and Pharmacology, UCLA School of Medicine, LA, CA; 3) Cardiovascular Research Center, MGH East, Harvard Medical School, Charlestown, MA.

DNA cytosine methylation in vertebrates influences many cellular events including gene transcription, genomic imprinting, and genome stability. The specific methylation pattern in a differentiated somatic cell is established through dynamic interplays of demethylation and de novo methylation during embryogenesis. We have previously demonstrated that proper DNA methylation is critically important for maintaining vital CNS functions and neuronal survival postnatally (Fan, et al., *J. Neurosci.* 21:788, 2001). However, whether de novo DNA methylation is important for CNS development has not been investigated. To explore the role of de novo DNA methylation in neural development, we compared the timing of neuronal and glial differentiation from wild-type and mutant embryonic stem (ES) cells that are deficient of de novo DNA methyltransferases Dnmt3a and Dnmt3b (Okano, et al., *Cell* 99:247, 1999). We established an in vitro differentiation protocol by which ES cells can be induced to differentiate into neural precursor cells (NPCs) that sequentially give rise to neurons and astroglial cells at different passages. When compared to the same passage of wild-type NPCs, Dnmt3a<sup>-/-</sup> and 3b<sup>-/-</sup> NPCs precociously gave rise to neurons and astroglial cells in early and late passages, respectively. Promoter reporter assays showed that neuron-specific (NeuroD) and glial lineage promoters (GFAP) are more active in Dnmt3a<sup>-/-</sup> and 3b<sup>-/-</sup> NPCs than that in control NPCs, suggesting that neurogenic and gliogenic program are prematurely activated in Dnmt3a<sup>-/-</sup> and 3b<sup>-/-</sup> NPCs. How de novo DNA methylation influences neurogenic and gliogenic program in differentiating NPCs will be further discussed.\*The first two authors made equal contribution to this abstract.

**BRCA1 and epigenetics in breast cancer.** *V. Birgisdottir<sup>1,2</sup>, S.K. Bodvarsdottir<sup>1,2</sup>, H. Hilmarsdottir<sup>1</sup>, J.G. Jonasson<sup>1,2</sup>, J.E. Eyfjord<sup>1,2</sup>.* 1) Icelandic Cancer Society; 2) University of Iceland, Reykjavik, Iceland.

Alterations in the BRCA1 gene have been linked to risk of breast cancer. Disfunction of the gene can be traced to germline mutations along with loss of the normal gene in tumor tissue. BRCA1 germline mutations are rare in Iceland; one such mutation has been found that explains <1% of breast cancer cases. The aim of this study was to assess whether epigenetic events, i.e. methylation of the BRCA1 promoter, might be of importance in sporadic tumors. Methylation of the BRCA1 promoter, which leads to loss of gene expression, was assessed in 130 sporadic breast cancer cases, using Methylation Specific PCR and gel electrophoresis. All tumors that showed methylation were analysed for allelic imbalance (AI) at BRCA1 and BRCA2 loci. AI was detected by amplifying microsatellite regions of normal/tumor DNA pairs and comparing peak allele sizes in an ALFexpress sequencer using Fragment analyzer software and also by FISH (fluorescent in situ hybridization). It has been suggested that mutation in the p53 gene is a necessary step in tumorigenesis in BRCA1 germline mutation carriers. Second aim was to search for p53 mutations in tumor samples that show methylation at the BRCA1 promoter. P53 analysis was carried out by DNA sequencing. Methylation at the BRCA1 promoter was found in 7.7% (10/130) of tumors tested supporting the hypothesis that methylation plays a role in functional inactivation of BRCA1 in sporadic tumors. 7/10 methylated tumors show clear AI and 2 tumors show borderline AI at the BRCA1 locus. These will be analysed further using FISH. AI at the BRCA2 locus was detected in 4/10 tumors and all those also show AI at the BRCA1 locus. These results show that the normal BRCA1 gene is frequently lost in tumors that have BRCA1 promoter methylation. Loss of both BRCA genes in 40% of methylated tumors indicates that both genes may be induced by, or function in, overlapping pathways in breast tumorigenesis. 40% of the methylated tumors carry p53 mutation. Our findings support the hypothesis that increased instability due to inactivation of caretaker genes such as BRCA1 leads to inactivation of important checkpoint genes like p53, which directly regulate tumor growth.

**Demethylation of the murine myogenin promoter is closely associated with transcriptional activation during development.** *T. Haines, P. Ainsworth, S-P. Yee, P. Bialek, D. Rodenhiser.* London Regional Cancer Centre and The University of Western Ontario, London, ON, Canada.

In many tissue-specific genes promoter methylation is correlated with transcriptional activity. Transcriptional activation is associated with hypomethylation while transcriptional repression is linked to promoter hypermethylation. We have examined the relationship between promoter methylation and transcriptional activity for the muscle specific gene myogenin. Using sodium bisulfite sequencing we have generated a developmental map of cytosine methylation for the murine minimal myogenin promoter from gamete to adult. Demethylation of the myogenin promoter is associated with transcriptional activation during development. At 7.5 days post fertilization the myogenin promoter is heavily methylated prior to transcriptional activation of myogenin around 8.5 days. Complete demethylation of the promoter is found in the developing somites of the 10.5 day embryo concurrent with the expression of myogenin. We found minimal methylation of the myogenin promoter in both gametes and the 2-cell preimplantation embryo suggesting that the promoter remains unmethylated until the genomic wave of *de novo* methylation occurs at implantation. We have demonstrated that tissue-specific methylation of the myogenin promoter in the adult mouse correlates with expression, with muscle showing the lowest levels of methylation while tissues of non-expression show higher levels of methylation. We have also examined tissue-specific methylation of an exogenous myogenin promoter in a transgenic mouse model. Methylation of the transgenic myogenin promoter was evaluated in muscle and brain at two time points in a myogenin-LacZ transgenic mouse line, initially while the gene was actively expressed in muscle, and again after the transgene had undergone silencing. The transgenic myogenin promoter was found to be heavily methylated in all cases where the transgene was not expressed and unmethylated only in muscle when it was active. Our results demonstrate that methylation of the myogenin promoter is inversely correlated with transcriptional activity in both the endogenous myogenin promoter and the transgene model.

Program Nr: 978 from 2003 ASHG Annual Meeting

**Epigenetic Control of hTERT Expression and Telomerase Activity in Differentiating Leukemia HL60 Cells.** *L. Liu, J. Berletch, J. Green, L. Andrews, T. Tollefsbol.* Univ Alabama at Birmingham 1300 University Blvd., CH175, Birmingham, Alabama.

The human promyelocytic leukemia HL60 cells display high telomerase activity, a phenotype related to their immortal status. All-trans retinoic acid (ATRA) was applied to induce the differentiation of HL60 cells. CD11b expression was examined to confirm the differentiation status of the treated cells. Decreased telomerase activity was observed in ATRA-treated HL60 cells, which is coupled with down-regulation of the hTERT gene. Upregulation of DNMT3a gene was observed during ATRA treatment, whereas no significant increase of DNA methylation in hTERT promoter. Acetylation of H3 is, however, dramatically decreased in the promoter region of hTERT, suggesting that downregulation of hTERT expression occurred through higher order of chromatin remodeling process. Our findings suggest that differentiation of HL60 cells requires inactivation of telomerase activity, which was achieved through epigenetic mechanisms controlling the expression of the hTERT gene.

**Frequent Epigenetic Inactivation of RASSF1A and BLu Genes Located within the Critical 3p21.3 Region in**

**Gliomas.** *L.B. Hesson<sup>1</sup>, D. Krex<sup>2</sup>, K. Hoang-Xuan<sup>3</sup>, E.R. Maher<sup>1</sup>, F. Latif<sup>1</sup>.* 1) Section of Medical and Molecular Genetics, Department of Paediatrics and Child Health, University of Birmingham, Edgbaston, B15 2TT, UK; 2) Department of Neurosurgery, Universitätsklinikum Carl Gustav Carus, Technische Universität Dresden, Fetscherstrabe 74, 01307 Dresden, Germany; 3) Federation neurologique Mazarin and Unite INSERM U495, Hopital de la Salpetriere, Paris, France.

RASSF1A is a major tumour suppressor gene located at 3p21.3. We investigated the role of aberrant promoter region hypermethylation of RASSF1A in a large series of adult gliomas. RASSF1A was frequently methylated in both primary tumours (36/63; 57%) and tumour cell lines (7/7; 100%). Hypermethylation of RASSF1A in glioma cell lines correlated with loss of expression and treatment with a demethylating agent reactivated RASSF1A gene expression. Next we investigated whether other members of the RASSF gene family were also inactivated by methylation. NORE1B and RASSF3 were not methylated in gliomas, whilst NORE1A and RASSF5 demonstrated methylation in glioma cell lines but not in primary tumours. We then investigated the methylation status of three other candidate 3p21.3 TSGs. CACNA2D2 and SEMA3B were not frequently methylated, but the BLu gene located just centromeric to RASSF1, was frequently methylated in glioma cell lines (7/7) and in 80% (35/44) of glioma tumours. In these tumour cell lines, BLu expression was restored after treatment with a demethylating agent. There was no association between RASSF1A and BLu methylation. RASSF1A methylation increased with tumour grade whilst BLu methylation was seen at similar frequencies in all grades. Our data implicates RASSF1A and BLu promoter methylation in the pathogenesis of adult gliomas, whilst other RASSF family members and CACNA2D2 and SEMA3B appear to have only minor roles. In addition BLu methylation is an early event in glioma development and that RASSF1A and BLu methylation appear to be independent and specific events and not due to region-wide changes in DNA methylation. In vitro and in vivo growth suppression studies in glioma tumor cell lines are in progress for both RASSF1A and BLu genes.

**DNA hypomethylation induces postnatal neuronal degeneration in the central nervous system.** *L. Hutnick, G. Fan.*  
Human Genetics, UCLA School of Medicine, Los Angeles, CA.

DNA cytosine methylation is a major epigenetic factor involving in gene regulation, genomic imprinting, and X-inactivation in development. Aberrant methylation has been attributed to a variety of human neurological disorders such as ICF and Rett syndrome. To understand the role of DNA methylation in neuronal development and function, we have generated CNS-specific conditional knockouts for the maintenance enzyme DNA methyltransferase I (Dnmt1). We crossed transgenic mice harboring floxed Dnmt1 allele with the Emx1-cre transgenic mice in which the cre-recombinase expression is restricted in the pallial cortical precursors that give rise to neocortex and hippocampus. Cre-loxP mediated Dnmt1 gene deletion occurred in precursor cells between E9-12, resulting in hypomethylation in postmitotic cortical and hippocampal neurons from the mid-gestation stage. Histological examination showed that mutant animals exhibited only very mild structural defect in cortex and hippocampus during embryonic and early newborn stages, suggesting that hypomethylation did not disrupt prenatal neurogenesis and cell differentiation. However, massive neuronal degeneration was evident in 2 to 3 weeks old mutant mice with a dramatic reduction of cortical and hippocampal structures. The loss of neuronal cells was accompanied by gliosis in these brain regions with a great increase in reactive astrocytes and activated microglial cells. Immunocytochemical analysis demonstrated that the remaining residual neurons in the mutant cortex and hippocampus were positive for parvalbumin, a marker of inhibitory interneurons that are not targeted by the Emx1-cre. Our results showed that DNA methylation is crucial for the survival of cortical and hippocampal neurons during the first three weeks of postnatal life--a critical window for CNS neuronal maturation. How DNA hypomethylation selectively induces postnatal CNS neuronal degeneration is currently under investigation.

**Environmental exposures, DNA methylation and breast cancer.** *D. Rodenhiser, B. Sadikovic.* London Regional Cancer Centre and The University of Western Ontario, London, ON, Canada.

Compromised patterns of DNA methylation result in genomic instability, altered patterns of gene expression and tumour formation. Specifically, aberrant DNA hypermethylation in gene promoter regions leads to chromatin condensation and gene silencing, while global hypomethylation changes can result in chromosomal instability and oncogene activation. Potential links exist between environmental agents and DNA methylation, but the destabilizing effects of environmental exposures on the DNA methylation machinery are not understood in the context of breast cancer etiology. We have assessed genome wide changes in methylation patterns and have identified gene sequences with altered methylation profiles in response to the common pollutant benzopyrene. A unique methylation profiling technique called amplification of inter-methylated sites (AIMS) has been used to generate easily readable fingerprints representing the cells DNA methylation profile. This novel protocol is based on the differential cleavage of DNA with isoschizomeric restriction enzymes having distinct methylation sensitivity. We have validated this approach by demonstrating both unique and reoccurring sites of genomic hypomethylation in four separate human breast carcinoma cell lines treated with the cytosine analog 5-azacytidine. Comparison of treated versus control samples reveals individual bands displaying methylation changes, and these bands can be excised, cloned, sequenced and the precise genomic location individually identified. In most cases, these regions of hypomethylation coincide with susceptible target regions previously associated with chromosome breakage, rearrangement or gene amplication. Similarly, we have observed that acute benzopyrene exposure is associated with altered methylation patterns in these same cell lines. These results reinforce a link between environmental exposures, DNA methylation and breast cancer and support a role for AIMS as a rapid, affordable screening method to identify environmentally induced DNA methylation changes that could initiate tumourigenesis. (Supported by the Canadian Breast Cancer Research Initiative.).

**Progressive epigenetic inactivation of RASSF1A in human mammary epithelial cells.** *M. Strunnikova<sup>1</sup>, G.P. Pfeifer<sup>2</sup>, R. Dammann<sup>1</sup>*. 1) AG Tumorgentik, Medizin. Fak., Martin-Luther-Universität, 06097 Halle/S., Germany; 2) Department of Biology, Beckman Research Institute, City of Hope Cancer Center, Duarte, California 91010, USA.

Epigenetic inactivation of the RASSF1A tumor suppressor gene by promoter hypermethylation was frequently detected in breast carcinoma. In this study, we investigated the mechanism of RASSF1A inactivation in normal human mammary epithelial cells (HMECs), which were grown for consecutive cell passages. In proceeding passages, the transcription of RASSF1A was highly reduced in the pre-senescent cells and a spreading of methylation into the RASSF1A CpG island from the flanking regions was observed. In parallel, the transcripts of the downstream RASSF1C and upstream BLU gene were significantly up-regulated. Furthermore, the p16INK4a tumor suppressor gene was epigenetically silenced in the post-senescent HMECs. In the post-senescent cells, the expression pattern of RASSF1A, RASSF1C, BLU and p16 was similar to the transcription levels observed in breast cancer cell lines. In conclusion, we observed drastic silencing of the RASSF1A tumor suppressor in proliferating HMECs and this inactivation was associated with progressive CpG island methylation, which may be triggered by promoter occlusion.

**CancerEpigenomics identifies a candidate tumor-associated gene.** *L.T. Smith<sup>1,2</sup>, C. Plass<sup>1,2</sup>*. 1) Department of Molecular Virology, Immunology, and Medical Genetics, The Ohio State University, Columbus, OH; 2) Division of Human Cancer Genetics, The Ohio State University, Columbus, OH.

Genetic changes resulting in DNA abnormalities including deletions, mutations and chromosomal anomalies are accepted determinants of cancer. In addition, epigenetic mechanisms, including DNA methylation, silence genes without altering their genomic sequence. Studies from our lab have demonstrated the utility of Restriction Landmark Genomic Scanning for identifying novel targets of DNA methylation on a genome-wide level. In the past, mainly genetic approaches guided searches for tumor suppressor genes. We hypothesize that by combining genetics with epigenetics (CancerEpigenomics) we can identify genes from regions of LOH by RLGS. BAC contigs were assembled spanning the chromosomal region, 6q23-6q24, for which LOH has been described in head and neck cancer squamous cell carcinomas (HNSCC) and non-small cell lung cancer (NSCLC). From this region, we identified 5 NotI and 5 AscI sites on the RLGS profiles. Detailed analysis of 18 tumor profiles demonstrated widespread aberrant methylation in this region, with a maximum peak at an AscI site located in the TCF21 gene. Upon RLGS analysis, TCF21 was identified as becoming aberrantly methylated in the majority of HNSCC, lung tumors, as well as lung cancer cell lines. To further assess the extent of TCF21 methylation, COBRA was performed, demonstrating that normal adjacent tissues are largely unmethylated, while tumor samples are aberrantly hypermethylated. Analysis in methylated patient samples and cell lines demonstrates reduced expression of TCF21. 5-aza-deoxycytidine treatment of cell lines resulted in reexpression, supporting gene silencing by DNA methylation. TCF21 functions in the transition from mesenchymal to epithelial cells in several developing organs. TCF21 knock-out mice die immediately following birth due to poor lung differentiation. However, the role of TCF21 inactivation in neoplastic disease has yet to be described. In summary, by combining common regions of LOH with RLGS, we have successfully identified a candidate gene that becomes altered in tumors by multiple mechanisms: genetically and epigenetically.

**Large fragile X premutation alleles may often contain two AGG interruptions.** *S.L. Nolin, G.E. Houck, A. Glicksman, A.D. Gargano, W.T. Brown.* Human Genetics Dept, Inst Basic Research, Staten Island, NY.

In the general population, the FMR1 CGG repeat is interrupted by AGGs, most commonly at positions 10 and 20. Males with premutation alleles are thought to contain one or no AGG interruptions within the repeat region. We have sequenced the region in 40 premutation males with >90 repeats and found that 25% have 2 AGG interruptions, 27.5% have 1 AGG and 47.5% have no AGG interruptions. Among 10 males with 2 AGGs, interruptions were observed at positions 10 and 20 from the 5' end. However, among 11 males with 1 AGG, 6 had an AGG at position 10, while 4 had an AGG at position 11. One additional male carried an AGG at position 12. The number of pure repeats inferred for 21 mothers of the males averaged 63, 69, and 72 CGGs for those with 2, 1 or no AGGs, respectively. The presence of 2 AGGs in the mothers appeared to offer some protection from expansion to full mutation in the offspring. For premutation transmissions from fathers, 8 (38%) daughters inherited a larger repeat, 10 (48%) inherited a smaller repeat and 3 (14%) inherited the same repeat size as their fathers. No apparent relationship to AGG patterns was evident. These studies suggest the role of AGG interruptions may have a greater importance in female than male transmissions and that there may be more than one path for repeat expansion.

**X inactivation skewing patterns change as women age.** *C.L. Anderson, C. Hatakeyama, C.L. Beever, M. Hayden, B. Casey, C.J. Brown, W.P. Robinson.* Dept Medical Genetics, University of BC, Vancouver, Canada.

While X chromosome inactivation (XCI) is generally thought to be a random process, most females exhibit some bias in terms of the frequency with which one or the other X is inactivated. Extremely skewed XCI (90% inactivation of one X) has been associated with various genetic diseases, recurrent pregnancy loss and trisomy risk, and has also been reported to increase as women age. This age-related increase has been suggested to be due to either selective differences between cells that have inactivated one X or the other, or to depletion of the stem cell pool with time. To further evaluate how XCI patterns change with age, we analyzed XCI status using a methylation assay at the AR locus in 332 women aged 0 to 88 with no known genetic disorders or abnormal pregnancy history. The incidence of extreme skewing increased with age, being observed in 8% of women under 20 years (N=75), and 24% of women over 60 years (N=89). Using a linear model, the average level of skewing increased from 66% at birth to 78% by age 80 ( $r^2 = 0.066$ ,  $p < 0.001$ ). A slightly better fit was observed with a quadratic model, with skewing increasing more rapidly later in life ( $r^2 = 0.068$ ,  $p < 0.001$ ), favouring a decrease in stem cell precursors as the explanation for increased skewed XCI with age. A surprisingly low concordance between the analysis of XCI using methylation assays at the AR and FMR1 loci was observed ( $r^2 = 0.287$ ,  $p < 0.001$ ). We hypothesize that this discordance reflects alteration of methylation, and are determining whether increases in skewed XCI are due to allele-specific methylation changes with age.

**A Coat Color Tagged Green Mouse with EGFP Expressed from the RNA Polymerase II Promoter.** *Y.C. Hsiao<sup>1</sup>, S.F. Lin<sup>1</sup>, C.Y. Tsai<sup>1,3</sup>, L.S. Horng<sup>1</sup>, H.H. Chang<sup>1</sup>, T.F. Tsai<sup>2,3,4,5</sup>*. 1) Dept of R&D, Level Biotechnology Inc., Taipei, Taiwan, Taiwan; 2) Department of Life Science, National Yang-Ming University, Taipei, Taiwan; 3) Institute of Genetics, National Yang-Ming University, Taipei, Taiwan; 4) Genome Research Center, National Yang-Ming University, Taipei, Taiwan; 5) Department of Medical Research and Education, Veterans General Hospital-Taipei, Taiwan.

To achieve visual genotyping for transgenic identification on the FVB/N albino background, we have successfully applied the tyrosinase cDNA to generate coat color tagged transgenic mice with high EGFP expression from the promoter of RNA polymerase II large subunit gene. The EGFP was expressed in more than 90% of the transgenic founders with the introduction of two copies of insulators. The green fluorescence was detected as early as the 1-cell fertilized egg stage and last for the whole embryo development. The EGFP was also expressed in all of the adult tissues examined with especially robust signal detected in the testis. Over-expression of EGFP may have toxicity to the testis leading to male sterile or decreased fertility. The coat color tagged green mice offer the opportunity for visual genotyping of newborns by dark eye pigmentation. The genotypes can be reconfirmed on postnatal day 7 by the light tan coat color that was clearly distinct from the albino coat.

**Genome-wide Analysis of Alternative Splicing in Human Tissues and Tumors.** *K. Mitsouras*<sup>1</sup>, *K. Lee*<sup>2</sup>, *Q. Xu*<sup>2</sup>, *C. Lee*<sup>2</sup>, *S. Nelson*<sup>1</sup>. 1) Human Genetics, UCLA, Los Angeles, CA; 2) Chemistry and Biochemistry, UCLA, Los Angeles, CA.

The goal of our studies is to investigate the pattern of alternative splicing in a subset of normal human tissues and, second, in a variety of brain tumor samples. We are developing a microarray platform that can specifically and sensitively detect tissue- and tumor-specific isoforms of human genes. We have used datasets of predicted alternatively spliced forms of human genes generated by computational methods to select candidates for printing on high-density oligonucleotide microarrays manufactured by Agilent Technologies. These arrays are designed to detect tissue- and tumor-specific isoforms of 142 human genes, and also contain probes for 30 yeast alternatively spliced isoforms from previously characterized yeast spliceosome mutants. Proof-of-principle experiments with yeast samples indicate that this technology can reliably pick up relative differences in the abundance of specific exons and splice junctions ranging from 3 to 20 fold. These experiments also suggest that an optimal probe length of 35-40mers is required to sensitively distinguish between splice isoforms. Preliminary experiments designed to compare the alternative splicing patterns between three normal human tissues (brain, spleen and liver) and a glioblastoma (GBM) sample revealed good correlation with the predicted alternative splicing events, especially in the brain vs GBM group. Many of these isoforms are in transcriptional regulators, or regulators of cell growth, thus implying that alternative splicing may play a role in tumorigenesis.

**Negative regulation of FOXC1 activity by the p44/p42 MAP kinase pathway.** *F. Mirzayans, F.B. Berry, R.A. Saleem, M.A. Walter.* Ophthalmology & Medical Gen, Univ Alberta, Edmonton, AB, Canada.

FOXC1 a member of the forkhead box transcription factor family of genes, plays an important role in ocular development. Mutations in FOXC1 result in Axenfeld-Rieger malformations, accompanied by secondary glaucoma in about 50% of cases. FOXC1 contains N- and C-terminal transcriptional activation domains. In addition, FOXC1 contains a 152 amino acid transcriptional inhibitory domain that when deleted results in a hyperactive FOXC1 protein and a decrease in its phosphorylation, suggesting an inverse relationship between FOXC1 phosphorylation and transactivation. There are 3 predicted p44/p42 MAP kinase sites in FOXC1. Therefore the effects of activation and inhibition of the MAP kinase pathway on FOXC1 ability to activate transcription were examined. Activation of this pathway in HeLa cells by PMA reduced FOXC1 transactivation by 5 fold, while inhibition with the MEK 1 and 2 inhibitor PD98059 stimulated FOXC1 transcriptional regulatory activity by 3 fold. Therefore stimulation of p44/p42 MAP kinase pathway results in inhibition of FOXC1 activity. However PMA treatment did not hamper the DNA binding ability of FOXC1, suggesting that the PMA induced FOXC1 defect was the result of impairment in the ability of FOXC1 to recruit transcriptional machinery. Interestingly, stimulation of the MAP kinase pathway has a stabilizing effect on FOXC1 protein levels. This stabilizing effect was not observed when cells were pre-treated with the inhibitor PD98059. The effect of mutating the 3 predicted MAP kinase phosphorylation sites on FOXC1 is being investigated. Together these data indicate that FOXC1 transcriptional activity is negatively regulated by p44/p42 MAP kinase activity. Such complex modulation of FOXC1 activity may be necessary for the proper execution of growth and differentiation programs.

**The role of *EYA1* and *EYA4* in deafness.** *Y. Zhang*<sup>1</sup>, *R.A. Friedman*<sup>2</sup>, *G. Van Camp*<sup>3</sup>, *R.J.H. Smith*<sup>1</sup>. 1) Department of Otolaryngology and Interdepartmental PhD Program in Genetics, University of Iowa, Iowa City, IA 52242, USA; 2) Department of Cell and Molecular Biology, House Ear Institute, 2100 West Third Street, Los Angeles, CA 90057, USA; 3) Department of Medical Genetics, University of Antwerp, Antwerp, Belgium.

The vertebrate *Eya* gene family is comprised of 4 transcriptional activators that interact with other proteins in a conserved regulatory hierarchy to ensure normal embryologic development. The structure of these proteins as deduced from their cDNA sequences includes a highly conserved 271 aa C-terminus called the *eya*-homologous region (*eyaHR*) and a more divergent transactivation domain at the N-terminus (*eya* variable region, *eyaVR*). Allele variants of *EYA1* and *EYA4* underlie two types of inherited human deafness, BOR syndrome and DFNA10, respectively. To clarify how mutations in these two genes impact the normal biology of hearing, we completed a number of functional studies. Using the yeast 2-hybrid system, we verified that *Eya1* and *Eya4* interact with *Six1*; we found no interaction with *Dach1*. As measured by -galactosidase activity, we found *Eya1-Six1* interaction to be stronger than *Eya4-Six1*. We next subcloned c-myc-tagged *Eya1HR* or *Eya4HR*, and HA-tagged *Six1*, and by immunofluorescence staining showed *Eya4* localization to the cytoplasm. After co-expression of *Six1*, *Eya4* was translocated to the nucleus. Results with *Eya1* were similar. Translation of mutant constructs (*Eya4*<sub>DFNA10</sub> and *Eya1*<sub>BOR</sub>) could not be demonstrated in transfected HEK 293 cells. We generated other truncated constructs of *Eya4* and also found that none localized to the cytoplasm. We were unable to demonstrate a translation product of mutant constructs by western blotting. Using a dual *Eya* construct (containing one wild type and a second one mutant or wild type allele), we confirmed no translation of the mutant allele, even if the mutation was non-truncating. These data suggest that haplo-insufficiency is the major cause of BOR and DFNA10. This research was supported by R01-DC03544 (RJHS).

**Expression of Degenerin BNaC/ASIC Isoforms in Hair Cells of the Mammalian Cochlea.** *H. Ostrer*<sup>1</sup>, *B. Fritsch*<sup>2</sup>, *T. Giudice*<sup>2</sup>, *R. Bing*<sup>1</sup>, *D. Hillman*<sup>1</sup>, *K. Beisel*<sup>2</sup>. 1) New York Univ Sch Medicine, New York, NY; 2) Creighton University School of Medicine, Omaha, NE.

The degenerins are a superfamily of genes that encode cation channels. The first degenerin cation channels to be identified were *mec4* and *mec10* in *C. elegans*, where mutations in the genes result in neurodegeneration. These subunits form complexes that are postulated to anchor to both sides of the membrane, producing mechanical tension that gates the cation channels. In mammals, two classes of degenerin ion channels have been identified, the epithelial sodium channels (ENaCs) that are expressed in kidney, colon and lung and the brain sodium channels (BNaCs), also known as the acid-sensitive ion channels (ASICs) that are expressed in different regions of the human and rat brain and spinal cord. Members of both the ENaC and the ASIC families are expressed in sensory neurons, suggesting that they could play a role in mechanically-gated ion touch receptors, including those that play a role in the mechanical transduction of sound waves in the cochlea. A role for ENaC was excluded because mice homozygous for a knockout mutation in this gene had normal hair cell function. By *in situ* hybridization, we have shown that members of the BNaC/ASIC family, ASIC and ASIC2, including their and splice isoforms, are expressed in inner and outer hair cells of the cochlea. By Western blot analysis, we have shown that these RNAs have been translated into proteins. By immunocytochemistry, we have shown that ASIC2 is expressed at tip links in hair cells of the cochlea. Despite their widespread expression in sensory neurons, members of the BNaC/ASIC family of degenerins may play a role in signal transduction in the cochlea.

**Generation and characterization of a DFNA5 knockout mouse.** *L. Van Laer<sup>1</sup>, M. Pfister<sup>2</sup>, S. Thys<sup>1</sup>, M. Mueller<sup>2</sup>, K. Vrijens<sup>1</sup>, L. Umans<sup>3</sup>, L. Serneels<sup>3</sup>, F. Kooy<sup>1</sup>, J.P. Timmermans<sup>4</sup>, F. Van Leuven<sup>3</sup>, G. Van Camp<sup>1</sup>.* 1) Department of Medical Genetics, University of Antwerp (Campus Drie Eiken), Antwerp, Belgium; 2) Hals-Nasen-Ohrenklinik, Universität Tübingen, Tübingen, Germany; 3) Experimental Genetics Group, K.U.Leuven, Leuven, Belgium; 4) Laboratory of Cell Biology and Histology, University of Antwerp (Campus Middelheim), Antwerp, Belgium.

A complex intronic insertion/deletion in DFNA5 is responsible for an autosomal dominant type of hearing impairment that segregates in an extended Dutch family. The hearing impairment starts in the high frequencies between 5 and 15 years of age and progressively affects all frequencies. On the mRNA level, the mutation results in exon 8 skipping. Despite extensive computational analysis, no putative function for DFNA5 could be deduced. In order to study its function *in vivo*, DFNA5 partial and complete knockout mice were generated by deleting exon 8 by targeted recombination in 129 ES cell lines to mimic the human mutation. An additional P-lox site was inserted before exon 7 to ensure the generation of a complete knockout mouse in case the deletion of exon 8 was insufficient. Because the 129 mouse strain exhibits early-onset hearing impairment, this genetic background was replaced by backcrossing for 6 generations into the C57Bl strain, which displays late-onset hearing loss, and into the CBA/Ca strain, which is a good-hearing reference strain. In homozygous DFNA5<sup>-/-</sup> mice, the presence of aberrant mRNA was demonstrated by RT-PCR. However, no protein could be detected using Western blotting, demonstrating that the DFNA5 gene was effectively silenced. To test the hearing impairment, frequency-specific ABR (Auditory Brainstem Response) was performed at different ages in the two genetic backgrounds. Morphological studies using scanning electron microscopy, complemented with organ of Corti explant studies, demonstrated significant differences in the number of fourth-row outer hair cells between DFNA5 knockout mice and their wild-type littermates. A complete histopathological analysis of 57 other organs revealed no additional abnormalities in the DFNA5 knockout mice.

**Missense CLDN14 proteins with defects in subcellular localization and inability to form tight junctions result in deafness.** *M. Wattenhofer*<sup>1</sup>, *A. Reymond*<sup>1</sup>, *A. Charollais*<sup>2</sup>, *D. Caille*<sup>2</sup>, *V. Falciola*<sup>1</sup>, *C. Borel*<sup>1</sup>, *A. Pampanos*<sup>3</sup>, *R. Rabionet*<sup>4</sup>, *M.B. Petersen*<sup>3</sup>, *X. Estivill*<sup>4</sup>, *P. Meda*<sup>2</sup>, *S.E. Antonarakis*<sup>1</sup>. 1) Division of Medical Genetics, University of Geneva Medical School; 2) Department of Morphology, University of Geneva Medical School; 3) Aghia Sophia Children Hospital, Athens; 4) Centre de Regulacio Genomica, Barcelona.

In developed countries, about 2/3 of the cases of isolated deafness have a genetic origin and about 50% of recessive cases are due to mutations in the GJB2 gene. Mutations in CLDN14 cause the DFNB29 deafness. This gene encodes a member of the claudin family, major components of tight junctions (TJ). To determine which CLDN14 function(s) was (were) altered in DFNB29, we studied the functional consequences of 2 missense substitutions associated with deafness. The first is the V85D substitution reported in a Pakistani family. The second is a G101R variant identified in a screen of CLDN14 ORFs in 183 non-syndromic deaf patients from Spain and Greece, negative for GJB2 mutations. A single chromosome was shown to harbor the 301G>A transition leading to G101R, which affects a conserved residue. At this position, part of the second transmembrane domain, all claudins examined have either a Gly (18/20 human CLDNs) or small hydrophobic residues (Ala or Val) but no arginine or other charged amino acid. R101 has been transmitted by the hearing father and cannot therefore be considered a highly penetrant dominant mutation. We screened the UTRs, for a potential maternally inherited mutation, in particular 3 newly-identified 5 exons and found no mutation. We ruled out large deletion through analysis of SNPs and microsatellites. This study indicates that mutations in CLDN14 do not substantially contribute to non-syndromic deafness in the Mediterranean population. The CLDN14 V85D protein shows a diffused cytoplasmic localization. In contrast, the CLDN14 G101R and WT proteins localize at the plasma membrane. However, LM cells transduced with the CLDN14 G101R show no formation of TJ unlike CLDN14 WT-transduced cells. These results suggest that the DFNB29 pathogenesis is due to, either subcellular mislocalization of the CLDN14 protein, or inability to form TJ.

**Development of a biochemical assay for the transporter function of ABCR.** *W. Wiszniewski<sup>1</sup>, J.R. Lupski<sup>1</sup>, T.G. Wensel<sup>2</sup>.* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Biochemistry, Baylor College of Medicine, Houston, TX.

Mutations of the ABCR gene are associated with many retinal phenotypes including Stargardt disease, Fundus Flavimaculatus, combined cone-rod dystrophy or retinitis pigmentosa. Some heterozygous mutations are associated with multifactorial disorder Age-related Macular Degeneration. The ABCR gene is expressed in the retina and encodes an ATP binding cassette transporter. This 220kD protein is exclusively found in discs of photoreceptors outer segments. The biochemical studies of ABCR and observations of mice lacking ABCR suggest that ABCR functions as a cytoplasm- directed flipase for N-retinylidene-PE (APE) that is a product of condensation of all-trans-retinal and phosphatidylethanolamine. The abnormally high concentration of APE in outer segments discs is considered to be the first step in pathogenesis of the ABCR associated retinal diseases. The aim of this study is to confirm directly the flipase function of ABCR and develop a biochemical assay for validation of the functional consequences of ABCR mutations. Our approach consists of the comparison of the transport rate of retinal initially conjugated to PE across the membrane of lipid vesicles containing wild type or mutated forms of ABCR using spectrophotometric analysis. The preliminary results showed that retinal in the form of APE localized in the internal layer of vesicles is transported slowly (many minutes) to the outer layer, likely as a result of slow Schiff's base hydrolysis rather than even slower spontaneous lipid flipping, so the acceleration of this process could have important significance in vivo. To test this hypothesis the vesicles containing ABCR proteins were formed and now are under investigations. The development of direct assay is crucial for a complete understanding of the role of ABCR in retinoid cycles and reliable validation of functional consequences of disease associated mutations found in patients with retinal diseases.

**Bridging structural biology and genetics by computational methods: An investigation into how the R774C mutation in the androgen receptor gene can result in complete androgen insensitivity syndrome.** *B. Gottlieb*<sup>1,2,3</sup>, *J.H. Wu*<sup>1,2,3</sup>, *G. Batist*<sup>1,2,3</sup>, *T. Sulea*<sup>4</sup>, *E.O. Purisima*<sup>4</sup>, *L.K. Beitel*<sup>1</sup>, *M. Trifiro*<sup>1,2,3</sup>. 1) Dept Cell Genetics, Lady Davis Institute for Medical Research, Sir Mortimer B. Davis-Jewish General Hospital, 3755 Cote-Ste-Catherine Rd, Montreal, Quebec, Canada, H3T 1E2; 2) Departments of Oncology and Medicine, McGill University, Montreal, Quebec, Canada; 3) Montreal Centre for Experimental Therapeutics in Cancer; 4) Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Ave, Montreal, Quebec, Canada, H4P 2R2.

Recent structural studies of the ligand-binding domain (LBD) of the androgen receptor (AR) have raised more questions than answers, as most of the known pathogenic mutations of the AR gene causing androgen insensitivity syndrome (AIS) are not in the putative ligand-binding pocket. In this study, we have investigated one such pathogenic mutation, by examining details of its altered atomic structure using a computational technique of molecular dynamics (MD) simulations extended over 4ns, effectively creating a 4D structural model. The mutation R774C which is in the LBD of the AR gene, causes complete AIS (CAIS) producing ARs that have a unique thermolabile profile, being thermostable at 22°C but thermolabile at 37°C. We have therefore investigated this mutation by MD simulations at 310 K (37 °C), 300 K (27 °C) and 293 K (20 °C). The MD simulations indicate that: i) the mutation causes local structural distortions, which result in changes in the shape of the ligand-binding pocket; ii) the mutation alters the dynamic nature of the protein and results in a more diverse conformational distribution of the ligand-binding pocket; and iii) the effect of the mutation on AR structure could be largely reversed by lowering the temperature at which the MD simulations were conducted. These results therefore strongly support the biochemical data, e.g., the mutants inability to form AR-ligand complexes at 37 °C and its characteristic reversible thermolability, clearly indicating the value of such computational methods.

**Identification of Similar Domains in Orthopoxvirus and Human Complement Regulators.** *J. Krushkal*<sup>1</sup>, *A. Emery*<sup>2</sup>, *E. Ciulla*<sup>2</sup>, *D. Konz*<sup>2</sup>, *R. Adkins*<sup>1</sup>, *I. Gigli*<sup>3</sup>. 1) University of Tennessee-Memphis, Memphis, TN; 2) Worcester Polytechnic Institute, Worcester, MA; 3) University of Texas-Houston, Houston, TX.

Genus Orthopoxviridae includes variola major virus that causes human smallpox. Many other orthopoxviruses are also important for public health, e.g. vaccinia, cowpox, monkeypox, camelpox viruses, and others. Genomes of orthopoxviruses encode for proteins with sequences similar to human regulators of complement activation (RCA), which allows these viruses to evade the host defense by downregulating the human complement.

We employed phylogenetic analysis to evaluate the structural relationships among SCRs of two types of orthopoxvirus RCA-like proteins and those of the human complement regulators, C4 binding protein chain (C4bp), membrane cofactor protein (MCP), decay accelerating factor (DAF), complement receptor type 1 (CR1), and factor H (FH). All complement regulators analyzed contain tandem short consensus repeats (SCRs). The first group of viral proteins analyzed included key regulators of complement activation in variola major virus (smallpox inhibitor of complement enzymes, or SPICE), vaccinia virus (vaccinia virus complement control protein, or VCP), and cowpox virus (inflammation modulatory protein, or IMP). These proteins were similar to N-terminal SCRs of MCP, DAF, CR1, and FH, and a number of additional SCRs. We provide a detailed list of similarity relationships between poxviral and human complement regulators. Sequence analysis suggested that orthopoxvirus complement regulators VCP, SPICE, and IMP arose from a single ancestral sequence that shared similarity with all human complement regulators.

The second group of poxviral proteins analyzed included plaque size-host range proteins B7R in variola virus, B5R in vaccinia virus, B4R in cowpoxvirus, and C1R in ectromelia virus. These proteins have function other than regulation of human complement. We found that these proteins have the order of their short consensus repeats different from that in functional complement regulators, and one of their SCRs, SCR 2, is very divergent from the functional regulators of complement activation.

**Cat eye syndrome critical region: Two overlapping mammalian genes within the *CECR6* mRNA? *I.M.D.***

*Mousseau, H.E. McDermid.* Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.

Cat eye syndrome (CES) is a rare genetic disorder caused by supernumerary (trisomic or tetrasomic) copies of the short arm and part of the long arm of chromosome 22. Symptoms for this rare condition are caused by the overexpression of one or more dosage-sensitive genes in the duplicated region and can include coloboma of the iris causing a "cat-like" pupil, heart defects, anal atresia, and renal and skeletal abnormalities. Mild mental retardation is also associated with this condition.

Analysis of the CES critical region (CESCR) by the McDermid laboratory led to the identification of 14 transcripts. Based on expression during development and the analysis of sequence motifs, we are presently concentrating on the functional analysis of three of these genes: *CECR1*, *CECR2* and *CECR6*. *CECR6* (Cat Eye syndrome, Critical Region, candidate 6) has several unusual features. The mRNA has the potential to code for two completely different novel proteins, which overlap in different reading frames. The smaller open reading frame (ORF, 627bp) starts two thirds into the larger ORF (1734bp) and their stop codons are less than 100bp apart. The predicted protein structure of the larger ORF is highly unusual, with numerous amino acid repeats such as poly-proline, poly-histidine, poly-glycine and poly-cysteine stretches. We are characterizing the structure and function of *CECR6*. Phylogenetic studies show that the larger ORF (578 amino acids) is conserved in mouse, rat, baboon and human, although no non-mammalian homologues have been found. The smaller ORF (209 amino acids) is only conserved in baboon and human (89% similarity) as the first methionine codon is not present in mouse or rat. We are conducting further phylogenetic studies in primates and other mammals to characterize the evolution of the small ORF sequence.

**Phylogenetic Footprinting of the *NF1* 5 Upstream Region (5UR).** *T.K. Lee, J.M. Friedman.* Medical Genetics, University of British Columbia, Vancouver, BC, Canada.

The 5UR of the human neurofibromatosis 1 gene was defined as the 59756 bp region between the *NF1* translation start site and the end of the first upstream GenScan prediction (NT\_010799.114). The 5URs of mouse and rat were defined as 59756 bp upstream of the translation start site of the *NF1* homologs in these species. The 5UR in *Fugu* was defined as the 1488 bp segment between the known 5 flanking gene (FN5) and the *NF1* translation start site. Sequence alignments were established by mVista, and windows of identity greater than that of the coding regions and extending 50 bp or more in length among all 3 mammalian species were identified with Frameslider, a Perl program written for this research. These highly homologous regions (HHRs) were compared to the *Fugu* 5UR using Pairwise BLAST and analyzed for potential transcription factor binding sites and other promoter-associated sequences with MATCH, MatInspector, Eukaryotic Promoter Database and TRRD.

Three HHRs were discovered in the *NF1* 5UR. HHR1, located 42626-42696 bp upstream of translation start site, contains an AP-1 site shared by all four species. HHR2, located 640-689 bp upstream of translation start site, has no promising predictions for recognized transcription factor binding sites. HHR3, located 233-519 bp upstream of the *NF1* translation start site, contains a previously-described CREB site that is shared by all three mammalian species.

HHR3 also includes a 24 bp sequence 310-333 bp upstream of the translation start that is identical in human, mouse and rat and differs by only 1 bp in *Fugu*. Bioinformatic analysis and correlation with previously-published in vitro transcription studies indicate that this sequence, which we call NF1 Highly Conserved Sequence (NF1HCS), is likely to be involved in transcriptional regulation. NF1HCS lies 151bp downstream from the *NF1* major transcriptional start site but appears to be a strong candidate for the *NF1* core promoter element despite its position further downstream than any previously-described eukaryotic downstream core promoter element.

Program Nr: 998 from 2003 ASHG Annual Meeting

**From ABC to ZNF, gene nomenclature and functional annotation.** *V.K. Khodiyar<sup>1</sup>, E.A. Bruford<sup>1</sup>, R.C. Lovering<sup>1</sup>, C.C. Talbot Jr<sup>2</sup>, M.W. Wright<sup>1</sup>, M.J. Lush<sup>1</sup>, S. Povey<sup>1</sup>, H.M. Wain<sup>1</sup>, HUGO Gene Nomenclature Committee (HGNC).* 1) The Galton Laboratory, Department of Biology, University College London, Wolfson House, 4, Stephenson Way, London, NW1 2HE, UK; 2) The Johns Hopkins School of Medicine, Institute of Genetic Medicine, The Johns Hopkins University, Baltimore, MD, 21287, USA.

Genome annotation is now the highest priority in the field of genomics and the HUGO Gene Nomenclature Committee (HGNC) is in an ideal position to assist in its provision. Our aim is to increase the ability of scientists to find and discuss information about genes by providing a unique, approved name and short-form symbol for every human gene. Assignment of approved nomenclature involves data analysis, reading the literature and corresponding with authors; information gathering which can be used for further annotation. Gene names can describe many characteristics including phenotype, domain structure, location, function and inter-species homology. However, the approved name can include only part of the wealth of data that may be known for a gene. It is the unique gene symbol (derived from the name) that is crucial in ensuring the association of related information e.g. functional Gene Ontology terms, with the correct gene record in both databases and the literature. We have named over 100 gene families and groupings from ABC to ZNF, and will present examples that demonstrate conservation of homology or function. Our close collaboration with the Mouse Genomic Nomenclature Committee has enabled synchronisation of the naming of human/mouse orthologous genes, and the sequencing of other genomes has provided increasing (and exciting!) help in annotation. 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 HUGO Booth.

**Assessment of accuracy of protein function prediction from gene expressions and protein-protein interactions.** Z. Tu, M. Deng, T. Chen, F. Sun. Molecular and Computational Biology, University of Southern California, Los Angeles, CA.

Several methods have been used to predict protein functions based on gene expression data and protein-protein interactions using the guilt-by-association principle. However, for gene expression data, not all the biological regulations can be observed at the transcript level. For some functional categories related to protein metabolism (rRNA processing, amine biosynthesis, amino acid metabolism and protein biosynthesis as defined in Gene Ontology (GO)), proteins within the same functional category have highly correlated gene expressions and these functions can be more accurately predicted using highly correlated gene pairs (HCGP). While functions of other categories cannot be accurately predicted since gene expressions are not significantly correlated. Protein-protein interactions including physical and genetic interactions have also been used to predict protein functions. Not all protein functional categories can be predicted with the same accuracy using different interaction datasets.

We develop a new statistical method to assess the accuracy of protein function predictions using gene expressions, protein physical and genetic interactions based on GO functional categories. We analyze three different microarray gene expression datasets (Rosetta compendium, yeast cell cycle and stress response) and MIPS physical and genetic interaction data. For all the three gene expression datasets, function categories related to protein metabolism can be more accurately predicted. A detailed analysis of the yeast stress response data shows that the set of functional categories with high prediction accuracy depends on experiment conditions. For example, when yeast are treated with H<sub>2</sub>O<sub>2</sub>, proteins having the ubiquitin-dependent protein catabolism function show significant levels of co-regulation, as opposed to untreated cells. We also apply this new method to study the prediction accuracy based on physical and genetic interactions. All the functional categories can be predicted with comparatively high accuracy for both interaction datasets with genetic interactions having the highest prediction accuracy.

Program Nr: 1000 from 2003 ASHG Annual Meeting

**Genome-wide search for 3'UTR motifs associated with mRNA turnover in Human T lymphocytes.** *L. Wang, X. Xu.* Program for Population Genetics, Harvard School of Public Health, Boston, MA.

The 3' untranslated regions of eukaryotic mRNAs are known to play an important role in post-transcriptional regulation of gene expression. Although several studies have determined mRNA turnover rate at genome scale with high-density DNA microarrays, few systematic studies on sequence motifs related to mRNA turnover have been reported. Raghavan et al. previously reported the turnover rate of approximately 6000 mRNAs in human T lymphocytes. Using this data set, we selected 215 fast-decay and 194 slow-decay mRNAs to conduct a systematic search for the sequence motifs in the 3' UTR that are associated with mRNA turnover. We compared the count frequencies in fast and slow decay mRNAs of all possible 7-nt motifs, repetitive sequences, as well as end sequences of 82 known human microRNAs. Furthermore, we searched for conservative motifs in both the fast and the slow decay mRNAs using a hidden Markov model implemented in the software BioProspector. The AT-rich repetitive sequences, which may contain the well-known AU-rich elements (AREs), were significantly more abundant in the fast decay mRNAs. No other repetitive sequences were found associated with the turnover rate. In addition, we found several novel elements that were associated with mRNA turnover. Specifically, we found GCCCNNG was associated with slow decay and WWUGUWW associated with fast decay. The latter was consistently detected by both the frequency counting and the HMM methods. We also found the occurrence of the reverse complement of the 5' end of human microRNA mir-106, ACUUUU, was significantly higher in fast decay mRNAs.

**Novel structure and functional peculiarities of human gene MOB.** *I.P. Vladychenskaya, L.V. Dergunova, V.G. Dmitrieva, S.A. Limborska.* Dept Human Genetics, Inst Molecular Genetics, Moscow, Russia.

Here we report new data on structure and functional peculiarities of novel human gene MOB. Transcript of 3734 nucleotides in length was predicted in silico for this gene. The 5-end of the coding region lies within the exon VII and the 3-end within the exon XI. 5-untranslated region of this transcript is represented by exons I-VI and partly exon VII. Exon I is rich in G and C and seems to be highly structured; three short ORFs are localized within the exons I, V and VI. This transcript was detected by means of RT-PCR as the major MOB gene transcript in human cerebellum tissue. Also less abundant alternative transcripts of MOB gene were revealed in the same tissue. We have determined the primary structure for the major transcript and for the smallest of the alternative transcripts. Nucleotide sequence of the major transcript was identical to that predicted in silico, the alternative transcript under study did not harbour the largest MOB gene coding exon (exon VII). MOB gene reported previously as brain-specific was shown to be expressed also in non-brain human tissues by means of semi-quantitative RT-PCR. The highest expression level of MOB gene was detected in human cortex, hippocampus and cerebellum. The comparable expression level was detected in kidney. The lowest expression level was demonstrated in spleen and lymphatic nodes.

**Regulated alternative splicing of an exon in the 3' end of a type V collagen gene, *COL5A1*.** A.L. Mitchell, U. Schwarze, P.H. Byers. Univ Washington, Seattle, WA.

Type V collagen is a heterotrimer of 2 pro1(V) chains (*COL5A1*), and 1 pro2(V) chain (*COL5A2*) or a pro1(V) homotrimer. Mutations in *COL5A1* result in classical Ehlers Danlos syndrome (EDS). While screening cDNA from individuals with EDS, we found that the sequence of exon 64 was heterogeneous as a result of alternative use of 2 exons, 64A and 64B. They differ at 35/69 basepairs and the proteins differ at 12/23 residues. The 2 exons are separated by an intron (IVS64A) of 63bps in humans and 61bp in the mouse and rat. IVS64A is too small to allow inclusion of both exons so only 1 version is included in each transcript. Upstream mutations that destabilize mRNA from one allele still result in production of both transcripts, indicating that alternative splicing is transcript specific and not allele-dependent. None of the known or our new mutations (W165X; G1489R; C1835S; IVS19+1GA; IVS27-1GA; IVS35+1GA; IVS35-2 AC; ins3680+T; del4916-4931) in *COL5A1* affect either exon. To determine if these exons are differentially used and a source of tissue variation, we analyzed RNA from adult mouse tissues. Although all tissues expressed both transcripts, the proportions differed dramatically. In many, the 2 isoforms were expressed in approximately equal amounts. However, testis, brain, and lung expressed predominately the A isoform. Computational folding of 380nt of precursor RNA identified several different structures that could present splice donor sites, a factor that could contribute to the preferential identification of 64A or 64B by the spliceosome. Exon 64 is just upstream of the region that encodes a domain involved in chain selection for fibrillar pro chains. We have cloned and expressed the cDNA coding sequences of the C-terminal domains (3' end of the mRNA) from the 3 type V collagen genes (*COL5A1*-the 64A and 64B isoforms separately, *COL5A2*, *COL5A3*). Co-expression studies to determine the interaction partners are underway and should identify the important domains for chain association. Tissue specific differences in expression are likely related to functional differences in the molecules, so mutations in these domains could have different phenotypes.

**A new ENU-induced allele of the mouse quaking gene causes severe central nervous system dysmyelination.** *J.D. Dapper*<sup>1</sup>, *J.K. Noveroske*<sup>1,2</sup>, *R.J. Hardy*<sup>3</sup>, *H. Vogel*<sup>4</sup>, *N. Box*<sup>1</sup>, *M.J. Justice*<sup>1</sup>. 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) The Jackson Laboratory, Bar Harbor, ME; 3) Division of Neuroscience, Imperial College School of Medicine, London, UK; 4) Department of Pathology, Stanford University, Stanford, CA.

The mutant allele series of the mouse *quaking* gene consists of the spontaneous  $qk^v$  allele that is homozygous viable with a dysmyelination phenotype, and four ENU-induced alleles ( $qk^{kt1}$ ,  $qk^{k2}$ ,  $qk^{kt3/4}$ , and  $qk^{l-1}$ ) that are homozygous embryonic lethal. Here we report the isolation of  $qk^{e5}$ , the first ENU-induced, viable allele of *quaking*. Unlike  $qk^v/qk^v$ ,  $qk^{e5}/qk^{e5}$  animals have early onset seizures, severe ataxia and a drastically reduced life span. Ultrastructural analysis of  $qk^{e5}/qk^{e5}$  brains reveals a severe lack of myelin when compared to both wild-type and  $qk^v/qk^v$  brains. In addition, detection of calbindin in the cerebellum of young adult  $qk^{e5}/qk^{e5}$  mice reveals Purkinje cell axonal swellings that are not reported in young adult  $qk^v/qk^v$  mice, indicative of neurodegeneration. Although the molecular defect in the  $qk^{e5}$  allele is not evident by sequencing or Northern analysis of quaking transcripts, protein expression studies show that two of the quaking protein isoforms, QKI-6 and QKI-7, are absent and QKI-5 is reduced in  $qk^{e5}/qk^{e5}$  postnatal oligodendrocytes. The oligodendrocyte developmental markers PDGFaR, NG2, O4, CNP and MBP are present in the  $qk^{e5}/qk^{e5}$  postnatal brain, with the later markers showing reduced levels suggesting an oligodendrocyte maturation defect may also be occurring.

**LERN1, a novel leucine-rich repeat protein with specific expression in the limbic system.** *L. Carim-Todd*<sup>1</sup>, *X. Estivill*<sup>2</sup>, *L. Sumoy*<sup>1</sup>. 1) Programme of Bioinformatics and Genomics, Centre de Regulació Genòmica (CRG), Barcelona, Catalunya, Spain; 2) Programme of Genes and Disease, Centre de Regulació Genòmica (CRG), Barcelona, Catalunya, Spain.

Using an *in silico* approach we have analyzed the gene content of human chromosome 15q24-q26. Among the several genes identified we focused on the molecular characterization of a novel leucine-rich repeat encoding gene, LRRN6A (Leucine-Rich Repeat Neuronal 6 A). We have confirmed the mapping of LRRN6A to 15q24, at the centromeric end of a highly complex region of susceptibility to panic and anxiety disorders. LRRN6A encodes a transmembrane leucine-rich repeat protein, LERN1 (Leucine-Rich Repeat Neuronal protein 1), with similarity to proteins involved in axonal guidance and migration, development of the nervous system and axonal regeneration processes. The identification of homologous genes to LRRN6A on human chromosomes 9 and 19 and of orthologous genes in the mouse genome and other organisms suggests that LERN proteins constitute a novel subfamily of LRR (Leucine-Rich Repeat) containing proteins. *In situ* hybridization with a mouse *Lrrn6A* probe reveals a specific central nervous system pattern of expression, highly and broadly expressed in the nervous system during somitogenesis stages (E10-E17) and gradually restricted to forebrain structures as development proceeds (E17 onwards). Overall levels of expression in adulthood are lower but remain constant and significantly enriched in structures belonging to the limbic system. Taken together, LRRN6A's inclusion in the 15q24-q26 region of susceptibility to anxiety disorders, its transcript distribution within the brain, its predicted protein structure and the homology of the LERN1 protein to other neural LRR proteins, suggest that LRRN6A is a potential candidate gene to contribute to the molecular and cellular pathogenesis of mental disorders.

**Loss of *Cecr2* causes neural tube defects.** *T.M. Ames, M.D. Kardel, H.E. McDermid, G.S. Banting.* Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.

Neural tube defects (NTDs), the second most common birth defect in humans, are caused by the failure of the neural tube to close during early development. NTDs are considered multifactorial in humans, however in mice the loss of many single genes have been shown to cause NTDs. We show here that loss of putative transcriptional coactivator *Cecr2* also causes NTDs in mice. We have created a knockout mouse using ES cells containing a genetrapp insertion within intron 7 of *Cecr2*. Mice homozygous for the genetrapp exhibit the NTD exencephaly, equivalent to anencephaly in humans, with a penetrance of 67% on a mixed 129P2:BALB/c genetic background. Nonpenetrant individuals are normal and fertile. LacZ reporter gene expression from the gene trap vector in heterozygous mouse embryos show *Cecr2* expression in the brain, spinal cord, spinal ganglia, nasal cavities, eyes and the digits of the limbs. Northern blot analysis of *CECR2* shows expression in most human adult tissues as well as human fetal brain, lung, and kidney.

*Cecr2* is a complex gene with the potential for multiple alternatively spliced transcripts. The protein sequence contains a bromodomain, nuclear localization signal, and AT hook, as well as several LXXLL-like binding motifs. The presence of these motifs suggests that *Cecr2* is a transcriptional coactivator, and therefore likely to be involved in chromatin remodeling and controlling the expression of other genes.

This mouse model will be useful for studying NTDs since exencephaly is the only phenotype shown to be caused by the loss of *Cecr2*. As *Cecr2* is likely a transcriptional coactivator, it may interact with other proteins already shown to cause exencephaly, thereby elucidating connections between such genes. Further characterization of *Cecr2* will shed new light on the mechanisms of neural tube closure.

***WHSC4* - a novel ncRNA gene in the Wolf-Hirschhorn syndrome critical region 1.** *S. Endele*<sup>1</sup>, *S. Schlickum*<sup>1</sup>, *N. Pfarr*<sup>2</sup>, *A. Winterpacht*<sup>1</sup>. 1) Institute of Human Genetics, Univ Erlangen-Nuremberg, Erlangen, Germany; 2) Childrens Hospital, University of Mainz, Mainz, Germany.

The Wolf-Hirschhorn syndrome (WHS) is a complex and variable malformation syndrome associated with the deletion of the terminal short arm of one chromosome 4. The WHS critical region has been confined to 165 kb in chromosomal subband 4p16.3. Recently, Zollino et al. (2003) suggested a second critical region (WHSCR2) immediately distal to WHSCR1. Clinical and cytogenetic data indicate that WHS is a contiguous gene syndrome, which means that haploinsufficiency of more than one gene in the affected chromosome region contribute to the phenotype. In order to elucidate the etiology and pathogenesis of this syndrome our studies aimed at identifying all genes and regulatory regions (in WHSCR1) contributing to the phenotype by comparative sequencing, computer assisted analysis, as well as functional studies. Two known and three novel genes (*WHSC1*, *WHSC2*, *LETM1*, *WHSC3*, *WHSC5*) were identified in the WHSCR1. In addition, we detected several EST matches as well as predicted exon sequences that did not correspond to any of these transcription units. By extensive RT-PCR analysis and rapid amplification of cDNA ends (RACE) as well as Northern blot analysis we could demonstrate that most of these sequences correspond to a single novel gene (*WHSC4*) which is expressed in a complex pattern in several tissues. *WHSC4* contains repetitive sequences, does not display any significant open reading frame and undergoes complex alternative splicing, leading to at least 4 different transcript classes (A-D). RACE experiments suggested at least 3 different transcriptional start sites and at least 3 different 3exons. The 5-end of one of these transcripts (A) overlaps with exon 1 of *WHSC3* on the opposite strand indicating that it might function as an antisense transcript. Other transcripts (B-D) span the entire *WHSC3* gene and/or overlap with parts of *WHSC2* in sense direction. All verified exons of *WHSC4* together span about 60 kb of the WHSCR1. The lack of significant open reading frames as well as its possible antisense function suggests a putative regulatory function of *WHSC4*.

**CAT 53: a protein phosphatase 1 nuclear targeting subunit encoded in the MHC Class 1 region may take part in memory and learning.** *J. Gruen*<sup>1</sup>, *R. Raha-Chowdhury*<sup>2</sup>. 1) Dept Pediatrics, Yale Univ Sch Medicine, New Haven, CT; 2) Centre for Brain Repair, University of Cambridge, Cambridge, UK.

CAT 53 is a phosphatase 1 nuclear targeting subunit (PNUTS), and a potent inhibitor of nuclear serine/threonine protein phosphatase 1 (PP1). It was identified by cDNA hybridization selection as an expressed sequence tag (EST) located in the vicinity of HLA-C and designated as CAT for HLA-C associated transcript number 53. It is a 99kDa protein expressed in several tissues including brain, muscle and liver. Fluorescence analysis with anti-CAT 53 antibody shows a punctate nucleoplasmic staining with accumulation in the nucleolus. In-situ hybridization studies of mouse brain show high expression in the olfactory cortex, piriform cortex and hippocampus, where CAT 53 is expressed in CA1, CA3, and mainly in the dentate gyrus. The hippocampus is important in development and plasticity, plays a fundamental role in learning and episodic memory, and has been implicated in a number of neurological and psychiatric disorders including Alzheimers disease, epilepsy, and schizophrenia. We present the genomic organisation of CAT 53, characterise the unique expression pattern in human brain, localize specific sites of mRNA transcription in thin sections of mouse brain by in-situ hybridization, and perform a structural analysis of the peptide domains.

**Characterization of Genes for the Calcitonin Gene Related Peptide (CGRP), its Receptor Component Protein (RCP) and the Generation of RCP Mouse Embryonic Stem Cells.** *L. Mnayer, I. Dickerson.* Physiology and Biophysics and The Dr. John T. Macdonald Foundation Center for Medical Genetics University of Miami School of Medicine, Miami, FL.

CGRP is a potent vasodilator neuropeptide secreted by central and peripheral nerves. Its receptor, the calcitonin receptor-like receptor (CRLR) signals through Gs and generates cyclic AMP. A receptor component protein (RCP) is necessary, but not sufficient for the formation of a stable receptor complex. We demonstrated that RCP was required for CGRP receptor signaling using antisense RNA to RCP expression in NIH3T3 cells. The reduction in RCP expression correlated with diminished CGRP-mediated signal transduction. Using co-immunoprecipitation we identified a complex of CRLR, RCP and a third accessory protein, receptor activity modifying receptor (RAMP-1) in NIH3T3 cells, guinea pig cerebellum and mouse embryonic stem cells (ES). Transcription start sites of the RCP promoter were identified using reverse transcriptase, ribonuclease protection assays and primer extension. The promoter is TATA-less, GC-rich and has four transcription start sites within a 50 bp region. We constructed an RCP target vector with 55% of the RCP gene replaced by green fluorescent protein, and neomycin phosphotransferase. RCP heterozygous ES cells were selected and confirmed by PCR, Southern blot analysis. A 50% reduction in RCP protein was observed in the heterozygotic cells. In conclusion we demonstrate the use of cell culture to determine the *in vitro* role of RCP in CGRP-mediated signal transduction. The generation of heterozygous knockout (RCP) ES cells should enable *in vivo* ascertainment of its functions.

**Fine mapping of duplication endpoints in Pelizaeus-Merzbacher disease.** *G. Hobson*<sup>1</sup>, *M. Cundall*<sup>2</sup>, *K. Sperle*<sup>1</sup>, *J. Kamholz*<sup>3</sup>, *J. Garbern*<sup>3</sup>, *H. Heng*<sup>3</sup>, *E. Sistermans*<sup>4</sup>, *S. Malcolm*<sup>2</sup>, *K. Woodward*<sup>2</sup>. 1) Dept Research, A I duPont Hosp Children, Wilmington, DE; 2) Inst Child Health, London, UK; 3) Wayne State Univ, Detroit, MI; 4) Univ Med Ctr Nijmegen, Netherlands.

Duplication of a portion of the long arm of the X-chromosome which includes the proteolipid protein 1 gene (*PLP1*) encoding the major central nervous system (CNS) myelin protein is the most common cause of the leukodystrophy Pelizaeus-Merzbacher disease (PMD). Animal studies have shown that overexpression of PLP1, a result of increased dosage of *PLP1*, can account for the disease pathogenesis, and that the severity of the disease is related to the amount of PLP1 overexpression. A study by Inoue and colleagues on 13 patients suggested that clinical severity of PMD is also related to the size of the duplicated segment. In this work we have investigated the size and location of the endpoints of the X-chromosome duplication in a cohort of 54 patients with PMD and a chromosomal duplication using a combination of interphase fluorescence in-situ hybridization (FISH), fiber FISH, quantitative PCR, and long range PCR. Although we find variability in the size of the duplicated region, from 100kb to 4.6Mb, we have not found a correlation between size of the duplicated region and clinical severity of the patients as assessed by a functional disability score developed for this purpose. Analysis of DNA sequence in the vicinity of duplication breakpoints does not demonstrate the presence of flanking low copy number repeats, suggesting that the chromosomal rearrangements are not mediated by non-allelic homologous recombination as commonly found in other genomic disorders. Recombination at the duplication breakpoints is likely caused by non-homologous end joining as proposed by Inoue and co-workers for deletions affecting this same chromosomal region. The molecular and genetic mechanisms underlying differences in disease severity, however, are not known, but are currently under investigation.

**Mutant ataxin-2 disrupts the Golgi complex and causes cell death.** *S.M. Pulst, D.P. Huynh, H. Ng, D. Nguyen, H.T. Yang.* Division of Neurology, Cedars-Sinai Medical Center-UCLA School of Medicine, Los Angeles, CA.

Spinocerebellar ataxia type 2 (SCA2) belongs to a class of neurodegenerative diseases caused by the expansion of the polyglutamine (polyQ) repeat. In contrast to other polyQ diseases, intranuclear inclusions of the SCA2 gene product ataxin-2 are not prominent in SCA2. In this report, we investigated the mechanisms underlying SCA2 pathogenesis using cellular models. COS1 cells and PC12 neurons were transiently transfected with plasmid constructs that express either GFP- or HA-tagged ataxin-2 proteins containing polyQ repeats of 22, 58, or 104 glutamines. Transfected cells were examined by confocal laser microscopy for morphological alteration, and cell death was determined by trypan blue exclusion assay, TUNEL staining, and caspase 3 enzymatic assays. In COS1 cell, both the endogenous and exogenous ataxin-2 proteins were localized in the Golgi apparatus. Ataxin-2 has an ER-exit signal and a trans-Golgi signal, and deletion of these motifs resulted in an altered subcellular ataxin-2 distribution. Expression of a full-length ataxin-2 with an expanded polyQ repeat disrupted the normal morphology of the Golgi complex and colocalization with Golgi markers was lost. Intranuclear inclusions were only seen when the polyQ repeat was expanded to 104 glutamines, and even then, were only observed in a minority of cells. Expression of ataxin-2 with expanded polyQ repeats in PC12 and COS1 cells increased cell death compared with normal ataxin-2, and elevated the levels of activated caspase-3 and TUNEL-positive cells. Cell death was completely inhibited by caspase-3 inhibitors. In conclusions, these results suggest a link between cell death mediated by mutant ataxin-2 and the stability of the Golgi complex. The formation of intranuclear aggregates is not necessary for cell death caused by expression of full-length mutant ataxin-2 in cultured cells.

**Investigation of the subcellular localisation of wildtype and mutant isoforms of spartin, mutated in a complicated form of HSP.** *H. Patel*<sup>1</sup>, *K. Whiting*<sup>2</sup>, *M.A. Patton*<sup>1</sup>, *A.H. Crosby*<sup>1</sup>. 1) Medical Genetics Unit, St. George's Hospital Medical School, London, UK; 2) King-George Laboratory, St. Georges Hospital Medical School, Cranmer Terrace, Tooting, London, UK.

The hereditary spastic paraplegias (HSPs) are a clinically diverse group of disorders in which progressive lower limb spasticity and weakness are primary features. We have shown that mutations in spartin underlie an autosomal recessive variant of HSP (Troyer syndrome, SPG20) present at high frequency amongst the Old Order Amish. Comparative sequence alignment revealed that spartin contains three protein domains (a MIT domain and two plant-related domains), one of which is also present in spastin, commonly mutated in autosomal dominant forms of HSP (SPG4). We have made a number of GFP-tagged constructs, both wildtype and three mutants including; (i) C-terminal portion deleted construct retaining only the N-terminal MIT domain, (ii) MIT domain deleted retaining only the plant-related regions, and (iii) a construct containing the 1110delA mutation identified in the Amish population, to evaluate the subcellular localisation of the spartin variants in the mouse neuronal cell line NSC34.

***WHSC5* - A novel candidate gene for neurological features of Wolf-Hirschhorn syndrome.** A. Winterpacht<sup>1</sup>, N. Pfarr<sup>1,2</sup>, S. Schlickum<sup>1</sup>, S. Richter<sup>1</sup>, B. Zabel<sup>2</sup>, S. Endele<sup>1</sup>. 1) Institute of Human Genetics, Univ Erlangen-Nuremberg, Erlangen, Germany; 2) Childrens Hospital, University of Mainz, Mainz, Germany.

The Wolf-Hirschhorn syndrome (WHS, MIM 194190) is caused by deletions in chromosome region 4p16.3 and is thought to be a true contiguous gene syndrome with a yet unknown number of genes contributing to the phenotype. Recently, several candidate genes have been described (*WHSC1*, *WHSC2*, *WHSC3*, *LETM1*) which are located in or immediately adjacent to the previously defined 165 kb WHS critical region 1 (WHSCR1). We have now focused our attention on the identification and functional characterization of genes which are involved in the neurological features (e.g. seizures, learning and motor deficiencies, ADHD) of the syndrome. Besides *LETM1*, which has become a strong candidate for seizures, we identified two further candidate genes by comparative sequencing and intensive database analysis (*WHSC3*, *WHSC5*). Here we describe the further functional characterization of *WHSC5*. *WHSC5* encodes a protein of approximately 300 aa in human and mouse. The protein most probably belongs to the superfamily of GCN5 related N-acetyltransferases (GNAT), showing the highest homology to members of the camello family of acetyltransferases. A member of this not very well characterized family has recently been implicated in early developmental processes. Northern Blot analysis revealed two main transcripts of approximately 3 and 6 kb in human and murine brain and testis. Whole mount and RNA in situ hybridization in early developmental stages of mouse development as well as in adult mouse brain showed that the expression is restricted to the neopallial cortex and the roof of the midbrain. Analysis of the *WHSC5* promotor has been performed and revealed putative target sites for transcription factors involved in development. The strong expression of the gene in fetal and adult brain tissues suggests an implication in brain development and/or function. Together with the predicted function in cell signaling and/or early developmental processes, *WHSC5* represents an excellent candidate gene for neurological features of Wolf-Hirschhorn syndrome.

**Characterization of an N-terminally Truncated WT1 Protein with Distinct Function Derived from an Alternative Promoter.** *A. Hossain, M. Nixon, C. Li, G.F. Saunders.* Department of Biochemistry and Molecular Biology, UT M. D. Anderson Cancer Center, Houston, TX 77030.

The Wilms tumor suppressor gene 1 (WT1) was originally identified as its association with Wilms tumor, a childhood nephroblastoma. In addition to causing Wilms tumor, mutations in WT1 often cause two distinct but overlapping urogenital defects in men Denys-Drash and Frasier syndromes. Alternative splicing of the WT1 transcript generated four major isoforms with different functional properties. We report here a previously unrecognized transcript originating from a second promoter that lies in intron 1 of the WT1 gene. This 2.5 kb WT11 transcript encodes a protein of about 35-37 kD depending on the alternatively spliced exon 5 and insertion/deletion of KTS amino acids in between the 3rd and 4th zinc-fingers. The N-terminal region of WT1 is known to contain the repressor domain and an RNA recognition motif encoded by exon 1. WT11 retains the intact DNA-binding and transactivation domain, but lacks the N-terminal 147 amino acids responsible for transcriptional repression. WT11 activates the Cyclin E promoter that is normally repressed by WT1. The expression pattern of WT11 is different from WT1 in different tissues. WT11 is highly expressed in adult testis, although low level expression can be detected in kidney, lung, spleen, and ovary. WT11 transcript is over expressed in almost all types of Leukemia, whereas full-length WT1 transcript is expressed in few cases. WT11 overexpression causes mesenchymal to epithelial conversions of SaOS-2 cells. Whereas, overexpression of full length WT1 causes cell death of SaOS-2 cells.

Program Nr: 1014 from 2003 ASHG Annual Meeting

**The role of transcription factor BTEB3 in the regulation of smooth muscle-specific gene expression in vivo.** *M. Keramatipour, P.D. Ellis, A.A. Grace, J.C. Metcalfe, P.R. Kemp.* Cardiovascular Biology Section, Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, United Kingdom.

Control of gene expression in vascular smooth muscle cells (VSMCs) is important in development and disease. During normal development smooth muscle  $\alpha$ -actin (SM  $\alpha$ -actin) is expressed prior to SM22a with smooth muscle myosin heavy chain (SM-MHC) expressed last. Dedifferentiation of these cells is a major pathological change seen in a number of conditions including atherosclerosis and restenosis after angioplasty. Recent evidence has implicated the Krppel like family of transcription factors (KLF family) in the control of SM-specific gene expression. BTEB3, a KLF identified in our laboratory, is widely expressed in both adult and embryonic mouse tissues. Our studies have implicated BTEB3 as a selective regulator of the smooth muscle-specific gene, SM22a, in VSMCs but not of either SM-MHC or SM  $\alpha$ -actin. To investigate the role of BTEB3 further we use two different methods to modify its expression in mice, standard knock out and siRNA transgenic mice. To make siRNA transgenic mice first two siRNA vectors were developed and showed that they knock down > 85% of BTEB3 expression in vitro. The siRNA construct was used to make transgenic mice and phenotype was analysed at different embryonic stages. Preliminary data showed that BTEB3 siRNA transgenic mice are not viable from ED 12 although the phenotype does not show full penetration. The effect of knock down of BTEB3 will be compared with the effect of knock out of BTEB3.

**Modulation of 5-hydroxytryptamine 4 (5HT4) receptor isoform expression predisposes to atrial fibrillation after coronary artery bypass surgery.** *S. Yusuf<sup>1</sup>, C. Fenske<sup>2</sup>, C. Dalageorgou<sup>2</sup>, N. Carter<sup>2</sup>, N. Al Saady<sup>1</sup>, N. Maarouf<sup>1</sup>, A.J. Camm<sup>1</sup>.* 1) Cardiovascular Medicine, St George's Hospital Medical School, London, UK; 2) Clinical Developmental Sciences, St George's Hospital Medical School, London UK.

5HT is a novel hormonal trigger to atrial fibrillation (AF), the commonest and most challenging of all the cardiac arrhythmias. 5HT is produced by serotonergic neurones in the central / enteric nervous systems and is stored / released by platelets when activated. It acts upon 5HT4 receptors on human atrial myocytes, which in turn are positively coupled to adenylyl cyclase, increasing heart rate. There are at least three different 5HT4 receptor isoforms resulting from alternate splicing and their exact role in atrial arrhythmias is not understood. We postulated that 5HT4(a) and (b) receptor isoform levels would be higher in patients susceptible to post coronary artery bypass grafting (CABG) AF and in those with the established arrhythmia (present pre and post operatively), the expression of both receptor isoforms would be down regulated due to electrical remodelling. Right atrial appendage samples were harvested from 52 patients undergoing bypass surgery. All patients had their pre operative underlying heart rhythm assessed and were followed up after the operation until discharge. 23 patients maintained sinus rhythm (SR) throughout; 14 patients developed AF post operatively and 15 patients had established AF. mRNA was extracted, purified and cDNA was synthesized using reverse transcriptase (Sensiscript Qiagen). Real time relative quantification PCR was carried out (Light Cycler Roche). Results showed ubiquitous 5HT4(a) and variable 5HT4(b) expression. A statistically significant reduction in 5HT4(a) expression levels in patients with established AF was shown (Student T-test  $p=0.001$ ). 5HT4(b) expression was higher in SR patients who developed post operative AF; this effect was even greater in patients with established arrhythmia compared to those who maintained SR throughout. Our study shows that expression of the 5HT4(b) receptor isoform predisposes to AF post CABG and also helps maintain AF.

Program Nr: 1016 from 2003 ASHG Annual Meeting

**CLN6 encodes linclin, a novel 28kD transmembrane protein.** *H. Gao, J. Espinola, ME. MacDonald.* Molecular Neurogenetics Unit, Massachusetts General Hospital, Harvard University, Charlestown, MA 02129.

The neuronal ceroid lipofuscinoses (NCLs) are recessive neurodegenerative disorders with lysosomal accumulation of autofluorescent membranous deposits. Mitochondrial ATP synthase subunit c is a major component of the deposits in the juvenile, late infantile and variant late infantile forms of the disease, suggesting that these are disorders of mitochondrial degradation. We have recently cloned CLN6, associated with Costa Rican vLINCL and have shown that disease stems from recessive mutations that alter the activity of linclin, a novel 311 amino acid transmembrane protein. To set the stage for studying linclin function in mitochondrial turnover, we are characterizing linclin. In vitro transcription and translation of CLN6. cDNA gives rise to a a 28kD polypeptide, that is not processed when microsomal membrane is added. We have used a lentiviral vector system to generate stable human brain cell lines that express linclin-V5 fusion protein. Immunoblot confirms an ~28 kD fusion protein, without evidence of stable processed isoforms. Confocal analyses with V5 monoclonal antibody reveals linclin in association with endoplasmic reticulum (ER), suggesting that linclin may be involved in steps of mitochondrial autophagy that require ER function. Our stable linclin neuronal cell lines, with others that express mutant linclin associated with vLINCL, will be valuable reagents for deciphering the activity of linclin in mitochondrial autophagy.

**Detection and characterization of beta-globin cluster rearrangements using Quantitative Multiplex PCR of Short fluorescent Fragments.** *S. Pissard*<sup>1,2</sup>, *J.P. Tabut*<sup>1</sup>, *Hao. Le Thi*<sup>4</sup>, *M. Goossens*<sup>1, 2</sup>, *T. Frebourg*<sup>3</sup>, *M. Tosi*<sup>3</sup>. 1)

Biochemistry and genetics, AP-HP, hop Henri Mondor, creteil, france; 2) INSERM U468 Hop Henri Mondor Creteil France; 3) INSERM EMI 9906, facut de Medecine et de Pharmacie, Universit de Rouen, Rouen, France; 4) departement of Human genetics, University of medicine and pharmacy, Ho Chi Minh city, Vietnam.

The beta-globin cluster contains approximately 50 percent of repeat sequences of various type, as revealed by Repeat Masker analysis and is therefore prone to deletion and recombination events. A great number of large deletions removing entirely or partially the globin gene and other globin locus genes have been described, which cause severe beta thalassemia disease. Since PCR is the common step of nearly all methods used for the molecular characterization of thalassemia, deletions are frequently not diagnosed and the real prevalence of such molecular defects is under evaluated. To make the detection of such events as easy as that of point mutations, we have designed a QMPSF (quantitative multiplex PCR of short fluorescent fragments) assay with a high density of test fragments around the b globin gene. We targeted 8 positions on the b globin locus : the LCR HS2, the gene, the A gene, the gene, the gene, the gene, two intergenic regions (inter and 3 of b gene) and a control gene not located on chromosome 11. We tested the method using samples displaying several known deletions (Chinese del: a thal, Sicilian del: thal, del -619) and recombination events generating hybrid genes like Hb lepore ( gene) and antilepore type ( gene). Our results were consistent with the expected deletion. However, using the antilepore sample, we found a pattern that does not correspond to the expected lepore counter-type recombination. This result would be in agreement with a gene conversion rather than a recombination event for the antilepore chain. This adaptation of QMPSF to the globin locus, provides a simple and reliable tool not only for detection but also for detailed characterization of the deletions or amplifications within the locus and should help to determine the real prevalence of these events and their molecular mechanisms.

**Norrie Disease Gene Mutations in Retinopathy of Prematurity.** *P. Paluru*<sup>1</sup>, *K. Hutchenson*<sup>2</sup>, *J. Koh*<sup>1</sup>, *E. Rappaport*<sup>1</sup>, *T. Young*<sup>1</sup>. 1) Ophthalmology, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Children's National Medical Center, Washington DC.

**PURPOSE:** Norrie disease is an X-linked recessive disorder characterized by bilateral retinal detachments, and variable hearing loss and developmental delay. Norrie disease gene (NDP) screenings primarily limited to exon 3 have been associated with heritable retinal vascular disorders, and in a small subset of patients with severe retinopathy of prematurity (ROP). We sought to correlate severity of ROP disease to NDP mutations in a large cohort of premature infants performing a mutation screen of the entire 3-exon gene. Parental carrier status was also evaluated.

**METHODS:** A total of 118 individuals of different ethnic backgrounds were consented and screened- 87 were pre-term infants and were 31 parents. Of the 87 infants, 52 had severe ROP (Stages 3-5). The median gestational age was 28.5 weeks (range 23-34), and the median birth weight was 1062.5 grams (range 367-1758). Seven amplicons spanning NDP were optimized for DHPLC and direct sequencing analysis. Three amplicons covered the coding region, and the remaining 4 spanned the 3UTR region.

**RESULTS:** Two sequence alterations were found, both in the 3UTR region of exon 3. A GA polymorphism at mRNA position 824 was found in an African American female and male with pre-threshold and threshold ROP, respectively. An AG polymorphism at mRNA position 1103 was found in an African American male and his normal mother.

**CONCLUSIONS:** Two novel nucleotide changes were found in the 3UTR region of exon 3 in our cohort. This is the first identification of NDP polymorphisms shown in African American infants who generally are less likely to develop severe levels of ROP. The results suggest that no association exists between NDP sequence alterations and severity of ROP in a large cohort of infants.

**Acknowledgements:** Blind Children's Center Foundation, Mabel E. Leslie Research Funds.

**Analysis of GJB2 heterozygotes for mutations affecting connexin 26 expression.** *R. Taggart*<sup>1</sup>, *E. Cohn*<sup>2</sup>, *P. Kelley*<sup>2</sup>, *W. Kimberling*<sup>2</sup>, *W. Sanger*<sup>3</sup>, *D. DiMaio*<sup>3</sup>, *M. Nelson*<sup>3</sup>, *J. Kenyon*<sup>3</sup>, *S. Smith*<sup>3</sup>. 1) Ctr Hearing & Deafness, SUNY Buffalo, Buffalo, NY; 2) Boys Town Natl Res Hosp, Omaha, NE; 3) University of Nebraska Med Ctr, Omaha, NE.

GJB2 (connexin 26) mutations are found in 20-30% of subjects with congenital nonsyndromic deafness, however as many as 30% have a single GJB2 mutation. The interpretation of a GJB2 heterozygote is difficult; hearing loss could be due to an undetected GJB2 mutation, an interaction with another gene mutation, or to an unrelated cause. One recently identified mutation is a 342 kb deletion (GJB6-D13S1830) that includes a portion of the coding exon of GJB6 (connexin 30), located 30 kb proximal to GJB2. The deletion includes the entire 5' upstream region of GJB6 and highly conserved noncoding regions that may coordinate expression of Cx26 & Cx30 within the inner ear. Hearing loss in subjects doubly heterozygous for the GJB6-D13S1830 deletion and a GJB2 mutation may be digenic inheritance, as a result of reduced expression of both Cx26 & Cx30. Alternatively, the GJB6-D13S1830 deletion may cause Cx26 related deafness by alteration of tissue specific Cx26 expression combined with loss of Cx30. We screened GJB2 heterozygotes with polymorphic markers in the region, FISH of metaphase chromosomes, quantitative PCR for gene copy differences and examined skin and retinoic acid treated fibroblasts for connexin 26, 30 and 43 expression. 101 deaf subjects with 2, 1 or no GJB2 mutations were examined with 11 polymorphic markers. Significantly increased levels of homozygosity ( $p < 0.0001$ ) were observed in subjects with 2 or 1 GJB2 mutations compared to subjects with no GJB2 mutations and controls. A GJB6 associated deletion was found in 2 of 21 GJB2 heterozygotes. No Cx26 expression was detected in skin from GJB2 35delG homozygotes, however reduced Cx26 expression was observed in retinoic acid induced fibroblast cultures from a subject heterozygous for the GJB6-D13S1830 deletion and the GJB2 35delG mutation. This suggests the GJB6-D13S1830 deletion removes regions that coordinate the expression of Cx 26 and 30. Screening for Cx26 expression in skin could prove invaluable to identify previously undetected mutations.

**Regulation of the nuclear translocation of the Carbohydrate Response Element binding protein, the Williams Beuren Syndrome Protein 14.** *G. Merla<sup>1</sup>, A.K. Stoeckman<sup>2</sup>, C. Howald<sup>1</sup>, S.E. Antonarakis<sup>1</sup>, H.C. Towle<sup>2</sup>, A. Raymond<sup>1</sup>.* 1) Division of Medical Genetics, Univ Geneva Med School, Geneva, Switzerland ; 2) Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota.

The Williams-Beuren syndrome (WBS) is a contiguous gene syndrome caused by chromosomal rearrangements at chromosome band 7q11.23. While our understanding of the etiology of WBS has improved greatly, the molecular basis of all except the cardiovascular phenotype remain unknown. Several endocrine phenotypes have been described for this clinically complex disorder, in particular impaired glucose tolerance (IGT) and silent diabetes.<sup>23</sup> Single copy loci map to the 1.5 Mb haploinsufficient region, while its flanking low copy repeat support multiple copies of 9 transcription units. Here, we study the potential involvement of one of the WBS genes, WBSCR14 in this pathology. The WBSCR14 gene encodes a member of the basic-Helix-Loop-Helix Leucine Zipper family of transcription factors, that dimerizes with the Max-like protein, Mlx, to bind E-box motifs in the promoter region of target genes. The rat ortholog of WBSCR14 is expressed under high glucose diet and inhibited under high fat diet in INS-1 pancreatic beta-cells. Its overexpression in primary cultured hepatocytes results in increased transcription of the LPK gene through direct binding of the Carbohydrate Response Element (ChoRE) of the LPK promoter. High glucose level activates also the nuclear translocation and the DNA-binding activity of rat WBSCR14. To identify new proteins that interact with WBSCR14, and potentially regulate its nuclear translocation, we performed a yeast two-hybrid selection and isolated four 14-3-3 isotypes and NIF3L1, that form a single polypeptide complex with WBSCR14 in mammalian cells. The active nuclear export of WBSCR14 is dependent upon the ability to bind 14-3-3, while its cytoplasmic retention is controlled by the NIF3L1 protein. Through these processes these interactors affect directly WBSCR14 transcription activation of lipogenic genes, as increasing amounts of NIF3L1 repress WBSCR14:Mlx ChoRE-driven transcription. We suggest a correlation between the WBSCR14 haploinsufficiency and the IGT observed in WBS patients.

**Complex Chromosome Rearrangement (CCR), 46,XY,t(5;6;20)(p13;p23;q13) in a male with oligospermia reproductive failure.** *D.S. Krishnamurthy, N. Al-Torki, S.A. Al-Awadi.* Cytogenetics Laboratory, Medical Genetics Ctr, Maternity Hospital, Kuwait.

Numerical and structural chromosomal abnormalities of autosomes and sex chromosomes are not uncommon in male infertility cases. Majority of structural rearrangements observed in constitutional cases originate from one or two break points (simple translocations, inversions, ring chromosome...). Complex chromosome rearrangement (CCR) occurring in phenotypically normal persons is rare. The rearrangement is considered to be balanced in phenotypically normal persons. CCR arise as familial or de novo mutation. A normal healthy couple (husband 29 yrs and wife 25 yrs) were investigated for secondary infertility and history of one spontaneous abortion (1st trimester). The husband's semen analysis confirmed oligospermia. The family history was unremarkable. Husband's seven brothers and 2 sisters are all phenotypically normal. Chromosome analysis of the wife confirmed normal 46,XX karyotype. Karyotype of the husband showed a balanced complex chromosome rearrangement (CCR), involving translocation of chromosomes 5;6;20 at break points, p13, p23 and q13 respectively. [46,XY, t(6;5)(p23;p13), t(5;20)(p13;q13)]. Heterozygote female carriers of CCR may be fertile and have pregnancies that produce phenotypically normal/balanced/affected children. However, male heterozygotes of CCR are often sterile or subfertile, because of spermatogenic arrest. The significance of CCR in male infertility will be discussed.

**Analysis of human-mouse conserved genomic sequences (Conserved Sequence Tags, CST) potentially involved in genetic diseases.** *S. Banfi*<sup>1</sup>, *D. di Bernardo*<sup>1</sup>, *A. Boccia*<sup>3</sup>, *A. Guffanti*<sup>5</sup>, *M. Petrillo*<sup>3</sup>, *S. Confalonieri*<sup>5</sup>, *F. Mignone*<sup>4</sup>, *G. Pesole*<sup>4</sup>, *C. Missero*<sup>1</sup>, *G. Paolella*<sup>2, 3</sup>, *A. Ballabio*<sup>1, 6</sup>. 1) TIGEM, Naples, Italy; 2) Department of Natural Sciences, University of Molise, Campobasso, Italy; 3) Biogem, Naples, Italy; 4) Department of Biochemistry, University of Milan, Milan, Italy; 5) FIRC Institute of Molecular Oncology, Milan, Italy; 6) Department of Pathology, Second University of Naples, Naples, Italy.

Comparative sequence analysis among genomes of different species represents an effective tool to identify conserved sequence elements that play a functional role in fundamental biological processes. The identification of conserved genomic regions surrounding disease-related genes is of crucial importance to better understand the cause of the disease and to gain important insights into mechanisms of gene regulation. We carried out a systematic genomic sequence comparison between 1000 human genes involved in the pathogenesis of genetic disorders and their murine counterparts. This analysis revealed the presence of over 60,000 sequence elements showing an identity greater than 75% between human and mouse over a region of at least of 100 base pairs. Approximately 65% of these sequences which we termed CSTs (Conserved Sequence Tags)- did not correspond to previously known exon sequences. We undertook a detailed bioinformatics analysis in order to gain insight into the possible role of these conserved sequences. In particular, we classified CSTs into the following subgroups: 1) transcribed CSTs (either belonging to coding or to non-coding transcripts); 2) non-transcribed CSTs with a high probability of representing functional elements, such as expression control elements; and 3) non-transcribed CSTs with a lower probability of having a functional role. These information, which will be entered into a publicly available CST database, should lead to a better comprehension of the biological role of CSTs and to a further elucidation of the mechanisms leading to human inherited diseases.

**Interleukin 31 is a novel four-helical-bundle cytokine that signals through a heterodimeric receptor complex expressed in epithelial cells of lung and skin.** *J.E. Parrish-Novak, C. Sprecher, A. Hammond, S. Presnell, J. Kuijper, F. Grant, Z. Gao, H. Haugen, S. Dillon, T. Whitmore, M. Franklin, M. Dasovich, M. Maurer, B. Harder, K. Waggle, T. Bukowski, D. Dong, P. Shea, D. Foster, J. Gross.* ZymoGenetics, Inc., Seattle, WA.

Four-helical-bundle cytokines have divergent primary sequences despite conserved secondary and tertiary protein structures. Cytokine receptors, however, share conserved primary sequence motifs readily recognized in sequence databases. Queries designed to detect these motifs identified a novel gp130-like receptor, *IL31RA*, in human genomic sequence. *IL31RA* is expressed in activated monocytes and T cells, and in epithelial cells in lung, skin, and prostate. *IL31RA* was expressed in a factor-dependent cell line along with other receptors in the gp130 class. Cells bearing *IL31RA* and OSM receptor responded to conditioned media from activated T cells. Functional cloning identified a novel cytokine, interleukin 31 (IL31). The intron phasing and exonic placement of structural elements in the *IL31* gene are conserved with those found in the genes encoding LIF, OSM, CT-1, and CLC; these related cytokines all activate gp130-class receptor complexes. In vitro assays using purified IL31 protein show activation of the Jak/STAT pathway in cells expressing both receptor subunits; expression of *IL31RA* alone is sufficient for binding but not for signal transduction. *IL31* is expressed in activated T and NK cells, with higher levels of transcript in Th2 than in Th1 cells. The genomic localization of the *IL31* gene to 12q24.3 is interesting in light of its characterization as a Th2 cytokine; this region includes a susceptibility locus for asthma, which is clearly Th2-driven. Overexpression of IL31 in transgenic mice resulted in a phenotype resembling human atopic dermatitis, also thought to be Th2-mediated. The transgenic phenotype is recapitulated with chronic delivery of pure IL31 to adult mice, demonstrating that it is not due to a defect in development. These data support a role for IL31 in Th2-mediated immune responses, and suggest that it may be a key cytokine in the pathogenesis of Th2-driven atopic diseases such as atopic dermatitis.

**Mouse PC7/LPC deficient embryonic fibroblast cell can process HIV gp160 protein.** *M. Ebihara*<sup>1,3,4,5</sup>, *D. Akanuma*<sup>2,4</sup>, *B.D. Young*<sup>3</sup>, *J. Meerabux*<sup>3,4</sup>, *Y. Sato*<sup>5</sup>, *T. Amagasa*<sup>2</sup>. 1) HMRO, The University of Kyoto, Kyoto, Kyoto, Japan; 2) Maxillofacial Surgery, Graduate School, Tokyo Medical and Dental University, Tokyo, 113-8549, Japan; 3) Medical Oncology Laboratory, Cancer Research UK, London, EC1M 6BQ, United Kingdom; 4) Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, Saitama 351-0198, Japan; 5) Department of Pathology IMCJ RESEARCH INSTITUTE, Tokyo, 162-0052, Japan.

Proprotein convertases (PCs) cleave proproteins to produce the mature form of regulatory proteins such as neuropeptides, growth factors, peptide hormones, enzymes and viral glycoproteins. There are now 7 known members of this family which includes furin, PC1/PC3, PC2, PC4, PC5/PC6, PACE4, and LPC/PC7/PC8/SPC7. Human immunodeficiency virus (HIV) glycoprotein (gp160) is processed by host cell proteases to produce gp120 and gp41. Recent study revealed that furin and/or PC7/LPC may involve in the processing of gp160. To investigate the role of PC7/LPC in the processing of gp160, we have produced knockout mice for the PC7/LPC gene, followed by establishing PC7/LPC (-/-) embryonic fibroblast cell line. Processing of gp160 was observed when the gp160 gene was transfected into PC7/LPC (-/-) cells, suggesting two possibilities, that is, (1) both the furin and PC7/LPC act as a processing enzyme for gp160 protein, (2) third enzyme exists as a major processing enzyme for gp160 protein.

**Promoter analysis of single-minded 2 (SIM2) and DSCR4 genes located on human chromosome 21q22.2. Y.**

*Shimizu<sup>1</sup>, A. Yamaki<sup>1</sup>, M. Uno<sup>1</sup>, S. Asai<sup>1</sup>, J. Kudoh<sup>2</sup>, S. Minoshima<sup>2</sup>, N. Shimizu<sup>2</sup>.* 1) Dept Medical Genetics, Kyorin Univ Sch Health Sci, Tokyo, Japan; 2) Dept Molecular Biology, Keio Univ Sch Medicine, Tokyo, Japan.

Human SIM2 gene is a homolog of *Drosophila* single-minded gene which plays a key role in the midline cell lineage of a central nervous system. To understand the molecular mechanism of SIM2 gene expression, we have analyzed the promoter region by transient transfection using a series of deletion constructs and point mutants fused with luciferase reporter gene and by gel retardation assay. We found that the c-myb like transcription factor cis-element located between nt-1351 and 1334 and the CAAT cis-element located between nt-1015 and -1004 appears to be important for SIM2 gene expression in T98G glioblastoma cells. Furthermore, we performed chromatin immunoprecipitation assay and found anti-C/EBPbeta but not anti-c-myb antibodies precipitated specifically the SIM2 promoter region between nt-1351 and 1004. We are in progress of identifying the protein which binds to c-myb like cis-element using the oligonucleotide beads. DSCR4 is expressed only in human placenta but its function is not known although the estimated protein has a low homology to TNF ligand family. We found DSCR4 is expressed in human placental choriocarcinoma cells BeWo. Using this cell line we determined the transcription starting site and analyzed the promoter region (about 2kb containing upstream of nt-94). We found OLF-1 cis-element between nt-790 and 760 has strong stimulative activity for DSCR4 expression and detected three specific bands consisting of OLF-1 cis-element and certain proteins in the gel retardation assay.

**Identification and analysis of the new, Vps13-like gene (V13LG) family that includes *CHAC*, the gene altered in chorea-acanthocytosis.** A. Velayos, A. Vettori, C. Dobson-Stone, A.P. Monaco. Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford, OX3 7BN, UK.

*CHAC* (9q21) was recently identified as the gene altered in Chorea-acanthocytosis, a rare autosomal recessive neurological disorder, whose characteristic features include hyperkinetic movements and abnormal red blood cell morphology. It encodes chorein, a large (3174aa) protein that shares significant homology with the yeast protein Vps13p, implicated in sorting of proteins like Kex2p. Comparison of chorein with the databases resulted in the identification of a number of human protein fragments not encoded by *CHAC*. We decided to analyse the genes encoding these proteins, as their study could lead to a better understanding of the function carried out by chorein. This analysis was performed as follows: i) deduction of the gene structures in silico, ii) verification of this structure by RT-PCR and sequencing, iii) analysis of the expression by northern blot and PCR in different tissues, iv) sequence comparison of the deduced proteins with each other and with homologous proteins in other organisms.

Three new *Vps13*-Like Genes (*V13LG2*, 3, 4), similar to *CHAC* (*V13LG1*) were identified, mapping on chromosome 15q21, 8q22 and 1p36, respectively. The main transcripts code for 3710, 4021 and 4362 aa proteins, respectively. *V13LG2* is highly similar to *CHAC*, suggesting a recent gene duplication; it also contains an internal duplication that explains the difference in size of both genes. Similarity of *V13LG3* protein with chorein is reduced to the N- and C-termini; this gene has been recently reported as *COH1*, involved in Cohen syndrome. *V13LG4* shows an intermediate similarity with *CHAC*. The four genes are widely expressed in human tissues, though several alternative splicing forms show difference in tissue distribution. Amino-acid sequence comparison shows the presence of two conserved regions at the N-terminus and the C-terminus of these proteins. These proteins could be involved in transport of different proteins, such as the proprotein convertases, homologous to the yeast Kex2p, which also constitute a gene family in higher species.

**Motoneuron Trophic Factor (MNTF) enhances peripheral nerve regeneration.** *D.L. Ash, D. Nussbaum, E.W. Jabs, T. Brushart.* Johns Hopkins Univ., Balto., MD.

MNTF is a 33 amino acid peptide identified from three wk old rat muscle extract electrophoresed on a 20% native PAGE gel. Thirty-four bands were resolved and excised (Chau et al 1992). Gel slices able to support survival of motor neuron colonies were selected for the production of polyclonal antibodies. These antibodies were used to screen a human retinoblastoma cDNA library. One plaque produced a novel transcript of 927bp that was localized to human chromosome 16q22 by use of a CHO/human hybrid chromosomal panel. Nucleotide BLAST of the transcript against the human EST database showed a 150 bp match, from normal adult human nerve tissue, which included the 99 bases that code for the MNTF human protein. We have found the human gene to be expressed in several tissues, including: human fetal thymus, liver, kidney; adult pituitary, 8-9 wk placenta, and weakly in the fetal skeletal muscle. To test function rat sciatic nerves were cut and the nerve endings sutured into the ends of a tube, leaving an 8mm gap between the ends. The enclosed gap was then filled with a solution of either 90% collagen and the MNTF protein, or collagen alone. Different concentrations of MNTF were tested by labeling the distal stump with the tracer Fluorogold, and counting the number of axons that managed to cross the gap within four wks. The results for each experimental group were: saline control, 540 axons;  $10^{-7}$ M MNTF, 687;  $10^{-6}$ M, 765;  $10^{-5}$ M, 873;  $10^{-4}$ M, 1111;  $10^{-3}$ M, 1130. We assessed whether MNTF had a specific effect on regeneration of motor versus sensory branches. End-to-end repairs of the rat femoral nerve were bathed in MNTF at the optimal concentration of  $10^{-4}$ M or in saline solution for three wks. Regeneration was evaluated by double-labeling the femoral muscle and cutaneous branches. With saline alone, a mean of 100 motor neurons projected correctly to muscle and a mean of 87 projected incorrectly to skin; a mean of 51 were double-labeled. After MNTF treatment, the mean number of motor neurons projecting correctly to muscle increased to 173 ( $p=0.0008$ ), with means of 59 projecting to skin and 47 double-labeled. Thus MNTF is present during development and has a specific effect on motor neuron regeneration with little impact on sensory projections.

**Cloning of the Human TSARG1 gene and the Mouse Mtsarg1 gene and the Expression Analysis in different development stages.** *J.J. Fu, L.Y. Li, X.W. Xing, S.F. Liu, G. Liu, G.X. Lu.* Human Reproduction & Stem Cell Institute, Central South University Xiangya Medical College, People's Republic of China.

Abstract: Applying the bioinformatics and experiment technique, we have cloned human and mouse novel gene cDNA sequences-human testis and spermatogenesis cell apoptosis related gene 1 (TSARG1) and mouse testis and spermatogenesis cell apoptosis related gene 1(Mtsarg1) from human and mouse testis cDNA library respectively, using a cDNA fragment (GenBank accession number: BE644538) as an electronic probe, which was significantly changed in expression in cryptorchidism in mouse by SSH technique. The GeneBank accession numbers of Mtsarg1 and TSARG1 are AF399971 and AY032925 (NM\_139073), respectively. The Mtsarg1 has a 55% identity and 61% similarity with TSARG1 at the amino acid level, which did not share significant homology with any other known protein in databases. The full-length cDNA of TSARG1 gene is 973 base pairs(bp)including 549 bp open read frame(ORF) and coding 183 amino acids, whereas the full-length cDNA of Mtsarg1 gene is 1103 bpincluding 576 bp ORF and coding 192 amino acids. The predicted molecule weight of TSARG1 is 19948.61 Daltonand the deduced iso-electric point is 10.24, whereas the Mtsarg1 is 20875.93 and is 9.83, being alkaline proteins. RT-PCR analysis showed that Mtsarg1 was expressed significantly in testis and faintly in epididymis in the ten tissues of testis, ovary, spleen, kidney, lung, heart, brain, epididymis, liver and skeletal muscle in mouse, while it wasn't expressed in the other eight tissues. Multiple tissues Northern blotting showed that Mtsarg1 expressed a 1.1kb fragment only in testis. To study the developmental regulations of the Mtsarg1 gene, RNAs were isolated from the testis of mice in the time of birth before, day 21, 35, 49, 280 and cryptorchid testis of day 14, and transported to the Nylon membrane. Northern blotting has been performed. Results showed that the Mtsarg1 transcript was detected lower signals in the time of day of 35 and higher in the time of day 49, 280 and cryptorchid testis of day 14, didn't detect any signals in the time of birth before, day 21 in testis in different development stages. Sex mature in mouse is about day 42 and indicated Mtsarg1 gene close related with the mouse spermatogenesis. Therefore, our results suggested that Mtsarg1 and TSARG1 would be pay important roles in the regulation of spermatogenesis cell apoptosis or spermatogenesis.

**Molecular identification of novel genes, especially transcription factors, from a human fetal growth plate cartilage library.** *S. Schlaubitz*<sup>1, 2</sup>, *C. Stelzer*<sup>1</sup>, *P. Hermanns*<sup>2</sup>, *A. Winterpacht*<sup>3</sup>, *T. Hankeln*<sup>4</sup>, *E.R. Schmidt*<sup>4</sup>, *F. Jakob*<sup>5</sup>, *B. Lee*<sup>2</sup>, *B.U. Zabel*<sup>1</sup>. 1) Children's Hospital, Mainz / Germany; 2) Dept. of Molecular and Human Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX; 3) Institute of Human Genetics, Erlangen-Nuremberg / Germany; 4) GENTERprise GmbH, Mainz / Germany; 5) Orthopedic Clinic, University of Wuerzburg / Germany.

We initiated a project aimed at the systematic identification and characterization of novel genes and the dissection of regulatory pathways involved in the complex processes of cartilage formation, growth, differentiation and homeostasis. The goal is to generate a number of candidate genes for inherited monogenic (skeletal dysplasias) as well as complex disorders (osteoarthritis / osteoarthrosis and osteoporosis) by sequence analysis of a fetal human cartilage cDNA library. So far, 5000 ESTs have been generated and analysed using the NCBI databases as well as the Ensembl Genome Browser (EMBL-EBI and Sanger) and the EST identification task ESTsweep provided by the HUSAR bioinformatics group at the DKFZ in Heidelberg/ Germany. By using the protein domain identification tool SMART we found potential transcription factors that are poorly characterized and performed electronic northern analyses. We selected those that show a specific expression in cartilage and / or bone, and have orthologous mouse and rat genes. At the moment, several ESTs are being characterized by RACE / RT-PCR analysis and expression studies using in-situ hybridization and Northern blotting. By construction of full-length cDNA libraries from different RNA samples (e.g. human fetal cartilage and human chondrocytes and osteocytes that were differentiated from mesenchymal stem cell culture) we will determine the complete cDNA sequence. These experiments will help us understand the influence of potential candidate genes in processes like pattern formation, ossification, postnatal growth and development of the skeleton.

**Characterization of cis elements and transcriptional determinants that confer tissue-specific expression of type X collagen gene during chondrogenesis in vivo.** Q. Zheng<sup>1</sup>, G. Zhou<sup>1</sup>, R. Morello<sup>1</sup>, D. Napierala<sup>1, 2</sup>, Y. Chen<sup>1</sup>, B. Keller<sup>1</sup>, X. Garcia-Rojas<sup>1</sup>, B. Lee<sup>1,2</sup>. 1) Molecular & Human Genetics,; 2) Howard Hughes Medical Institute, Baylor College of Med., Houston, TX.

Type X collagen (*Col10a1*) is the only known hypertrophic chondrocyte-specific molecular marker. Its deficiency causes Schmid metaphyseal chondrodysplasia (MCDS). Until now, cis elements directing its hypertrophic chondrocyte-specific expression in vivo have not been described. Our studies show that a 4-kb murine *Col10a1* promoter can drive -galactosidase expression in lower hypertrophic chondrocytes in transgenic mice, and *Runx2*, a Runt domain transcription factor, contributes to the transactivation of this promoter via conserved *Runx2* binding sites. *Col10a1* expression was decreased in *Runx2*<sup>+/-</sup> and was barely detectable in *Runx2*<sup>-/-</sup> mice limbs by real-time RT-PCR analysis. Moreover, *Runx2* showed two-fold up-regulation in hypertrophic MCT cells in which *Col10a1* was concurrently upregulated. Thus, *Runx2* directly regulates the activation of the type X collagen gene during chondrocyte maturation *in vivo*. However, this 4 kb *Col10a1* promoter containing RUNX2 elements can only drive weak reporter gene expression in the lower hypertrophic zone of transgenic mice. To identify additional cis-regulatory regions, we generated transgenic reporter mice for further promoter analysis. An 8 kb *Col10a1* regulatory region encompassing the same 4 kb proximal promoter and second intron can direct reporter gene expression throughout the zone of hypertrophy. In another transgenic mouse line containing a 10 kb regulatory element that adds an additional 2 kb 5' region to the 8 kb construct, the tissue-specific expression of transgene is even stronger than those of the 8 kb *Col10a1* sequence that directs expression throughout the hypertrophic zone. These in vivo data suggest the presence of strong enhancer elements in both *Col10a1* distal 5' promoter and intron 2 that cooperate with the Runx2 binding elements in the proximal promoter to specify high level expression throughout the hypertrophic zone of long bones. It will be important to determine if mutations in corresponding *COL10A1* regions can also causes MCDS.

**Expression of the PAX7 protein isoforms is regulated by alternative termination of transcription.** *E. Vorobyov, D. Horst, B. Dworniczak, J. Horst.* Institut fuer Humangenetik, Universitaetsklinikum, Muenster, Germany.

Expression of the PAX7 gene is known to be associated with determination of myogenic cell lineage in development of vertebrates. Recent data obtained in the Pax7 K/O-mice revealed its exclusive function in specification of muscle satellite cells during post-natal period (Seale et al., Cell 102, 2000). The fact that PAX7 is expressed in fast proliferating embrional myoblasts and in quiescent satellite cells of adults raised the question whether different PAX7 protein isoforms may have distinct roles in these myogenic precursors. In our previous study we have identified a PAX7 isoform expressed in human tumor of striated muscle, rhabdomyosarcoma (Vorobyov et al., Genomics 45, 1997). The C-end of this protein encoded by the last portion of exon 8 does not show any homology to the PAX7 orthologous genes of mouse, chicken, zebrafish and lamprey. In order to verify that this isoform of PAX7 is not an artificial product we extended the study of 3-ends of the Pax7 mRNAs in human and mouse. As a result, two main transcripts of Pax7 were identified in both organisms. The long Pax7 cDNA represents the complete protein ended with the conservative C-peptide, encoded by exon 9. The short cDNA results from premature termination of transcription caused by the presence of weak affinity termination signals in intron 8 of this gene. This truncated cDNA encodes a different C-end of the PAX7 protein. The existence of both protein isoforms was shown by immunoblotting with the PAX7-specific monoclonal antibody. We speculate that regulation of the Pax7 alternative termination of transcription may be dependent on the changes in concentration of termination-specific factors (e.g. the cleavage stimulating factors, CstF) that varies in different cell states. Finally we established stable cell lines with ectopic expression of these two Pax7 isoforms and performed a comparative analysis of the gene expression profiles in the corresponding cells by means of the Affymetrix gene chip system. Certain differences in expression of the cell cycle and extracellular matrix genes were identified that can be considered as candidates for future analyses of the PAX7 downstream regulatory pathways.

Program Nr: 1032 from 2003 ASHG Annual Meeting

**Overview of the signalling cascades of PTPN11, mutated in Noonan syndrome.** *G. Sivapalan, A. Crosby, S. Jeffery, K. Kalidas, A. Shaw, M. Patton.* Medical Genetics Research, St George's Hospital Medical School, London, UK.

Noonan syndrome is an autosomal dominant disorder characterized by dysmorphic facial features, short stature and a congenital heart defect, predominantly pulmonary stenosis. We have previously mapped the gene for Noonan syndrome to chromosome 12q24.3. As part of a collaborative group we recently identified mutations in the PTPN11 gene, which encodes a non-receptor protein tyrosine phosphatase (SHP2), in patients with Noonan syndrome. SHP2 is a ubiquitously expressed molecule which appears to play a role in a number of cell signalling and developmental pathways including the Ras-Raf-MAPK and JAK/STAT cascades. We have subsequently shown that Noonan syndrome is heterogenous and that mutations within PTPN11 account for ~40% of cases. In the absence of a single large pedigree unlinked to PTPN11 for linkage analysis, a candidate gene approach offers a possible strategy for the identification of a second causative gene. Consequently we have produced a comprehensive evaluation of the SHP2 signalling cascades and evaluated a number of genes in these pathways for disease associated mutations.

**Cloning and characterization of human zinc transporter 1 (*ZNT1*).** V.A. Coronado<sup>1</sup>, M.S. Nanji<sup>1</sup>, R.D. Palmiter<sup>2</sup>, D.W. Cox<sup>1</sup>. 1) Dept Medical Genetics, Univ Alberta, Edmonton, AB, Canada; 2) Dept Biochemistry, Univ Washington, Seattle, WA, USA.

Zinc is an essential metal, required for the proper function of several enzymes. However, when present in excess, zinc can become toxic to cells. Many of the pathways involved in zinc homeostasis have been studied in yeast, which use at least three different mechanisms to maintain zinc homeostasis. First, zinc-uptake transporters, Zrt1 and Zrt2, are under transcriptional regulation, being induced in zinc-limiting conditions. Secondly, these same transporters are then inactivated post-translationally by high zinc concentrations. The third mechanism of zinc homeostasis, in which Zrc1, Cot1, and Zrt3 have been implicated, is the sequestration of zinc in the vacuole, a suggested site for storage and detoxification under steady states of excess zinc. In mammals, zinc homeostasis is maintained by the SLC30 family of transporters, which has nine members, Znt1-9. Zinc is the only metal known to be transported by these proteins and counterparts of the SLC30 family are found in all organisms. We have cloned the human zinc transporter 1 (*ZNT1*) cDNA and show that the predicted protein has 85% amino acid identity to mouse and rat ZnT1. Northern blot analysis shows that *ZNT1* is expressed as a transcript of 2.4 kb and 7.5 kb in multiple tissues with highest expression in the kidney and liver. The gene spans a region of approximately 4 kb and consists of two exons separated by a 1.7 kb intron. The 5-prime region of the gene has a 73% GC content and two potential metal response elements for regulation. *ZNT1* was mapped to human chromosome region 1q32-41, using a human/hamster radiation hybrid panel. The *ZNT1* clone was unable to complement a *ZRC1* knockout in yeast, but did confer resistance to a zinc-sensitive BHK cell line lacking functional ZnT1. Mutation analysis of *ZNT1* in two patients with liver cholestasis associated with high hepatic zinc concentrations showed no mutations in the coding region or splice junctions.

Program Nr: 1034 from 2003 ASHG Annual Meeting

**Characterization of mammalian cell mutants with altered PCNA.** *N.C. Mishra, K. Taylor.* Biological Sciences, The University of South Carolina, Columbia, SC.

Mutants of Chinese hamster cells resistant to aphidicolin, DNA polymerase-inhibitors, were selected to gain insight into mechanism of DNA replication. Such mutants earlier were found to possess changes in alpha-family DNA polymerases. PCNA genes were cloned and encoded proteins were further characterized. The mutant PCNA showed substitution of nucleotides at three different positions in the sequence of the gene and in the (inferred) amino acid sequence of the protein encoded by the cloned gene. The mutant PCNA was further found to be unable to interact with CDK2 in a pull down assay, CDK2 is an important protein of the cell cycle. The mutant PCNA was also found to show its altered activity and binding to FEN1, an important protein involved in the removal of Okazaki fragments during eukaryotic DNA replication. The significance of these results will be discussed.

Program Nr: 1035 from 2003 ASHG Annual Meeting

**A novel alternative splicing isoform of the human FGD1 gene.** *T. Kaname, K. Yanagi, K. Naritomi.* Dept Medical Genetics, Univ Ryukyus, Nishihara, Japan.

*FGD1* gene is a responsible gene for facio-genital dysplasia (Aarskog-Scott syndrome, MIM No.305400.) The gene encodes a guanine nucleotide exchange factor (GEF), which activates the Cdc42, and the products affect cell morphology *in vitro*.

We have found a new splice-variant of the human *FGD1* gene, which is generated by alternative splicing of parts from intron 8 as a novel exon 8B. The new exon 8B consists of 59 nucleotides which causes frame shift of the authentic transcript. The predicted product from the alternative form has only Proline-rich domain and GEF domain, but lacks pleckstrin homology domains and a FYVE-finger domain. This truncated product will inhibit filopodia formation, in contrast to the wild type of product has such activity *in vitro*. The alternative form is expressed in the lung and kidney and also in transformed B cells.

**Cloning and characterization of a murine orthologue of NYX responsible for complete congenital stationary night blindness (cCSNB).** *M.A. Sarna, N.T. Bech-Hansen.* Medical Genetics, University of Calgary, Calgary, Alberta, Canada.

The complete form of X-linked CSNB (cCSNB) manifests in patients with clinical symptoms of impaired night vision, reduced visual acuity and in some cases myopia, nystagmus, and strabismus. The gene responsible for cCSNB has been identified as *NYX* and positioned to the X chromosome (Xp11.4). Greater than 80% of *NYX* mutations in patients with cCSNB are missense mutations. The *NYX* gene encodes nyctalopin, a GPI-anchored, leucine-rich protein of 481 amino acids. In this study, the mouse *Nyx* gene was identified and sequenced by using mouse BAC clones which had been obtained by screening with a human *NYX* probe. Subsequently, the mouse BAC clones were used in co-positioning *Nyx* and *Cask*, *CASK* is positioned to Xp11.4, on the mouse X chromosome. The mouse nyctalopin protein is predicted to be 476 amino acids in length and shares 84.2% identity with human nyctalopin. Similarly, the rat *NYX* gene has been located on the rat X chromosome. Using genome sequence (Accession: NW 044417.2) as well as EST clones, rat *NYX* was found to encode a protein of 476 amino acids, which shares 96% and 83.8% identity with the mouse and human nyctalopin proteins, respectively. In all three species, the *NYX* gene appears to be organized into three exons with exons 2 and 3 contributing to the open reading frame. These two exons are separated by a 25.5, 18.8 and 17.8 Kb intron in human, rat and mouse genes, respectively. The rodent nyctalopin proteins have a leucine-rich domain and a signal peptide sequence similar to that seen in the human protein but neither the mouse nor the rat nyctalopins are predicted to be GPI anchored, based on predictions using the available GPI-anchoring programs. Analysis of mRNA expression in mouse tissue, using the RNase protection assay, showed distinct *Nyx* expression in the eye and very low levels of expression in the brain, heart and lung. In contrast to the human *NYX* gene, which was shown to be expressed robustly in kidney, in addition to the retina, no expression was seen in mouse kidney tissue. On-going studies are aimed to elucidate the roles that nyctalopin plays in the neural circuitry of the visual system.

**Complementary genetic and functional analyses of SNPs in the extracellular domain of the human leptin receptor gene (LEPR).** *D.W Meechan*<sup>1,2</sup>, *N.D. Quinton*<sup>3</sup>, *R. Eastell*<sup>4</sup>, *A.I.F. Blakemore*<sup>2</sup>. 1) Biomedical Research Centre, Sheffield Hallam University, Sheffield, United Kingdom; 2) Department of Medical and Community Genetics, Imperial College London, St.Marks hospital, Watford road, Harrow, HA1 3UJ, United Kingdom; 3) School of Biomedical Sciences, University of Leeds, St.James Hospital, Leeds, United Kingdom; 4) Division of Biomedical Sciences, University of Sheffield, Northern General Hospital, Sheffield, S5 7AU, United Kingdom.

Previous studies have demonstrated an association between the GLN223ARG SNP of the human leptin receptor gene (LEPR) and indicators of adiposity in a cohort of postmenopausal Caucasian women. Subjects homozygous or heterozygous for the A allele (which codes for the GLN amino acid) have significantly higher BMI than those not carrying the A allele ( $p=0.007$ ). Further studies involving the same group indicated an association with the LYS109ARG SNP in the same gene. Individuals that are homozygous or heterozygous for the A allele, which codes for the LYS amino acid, have significantly higher BMI than those not carrying the A allele ( $p=0.017$ ). Linkage disequilibrium was observed between the two SNPs ( $D = 0.64$ ). To determine the functional significance and relative effects of the two SNPs, a series of LEPR extracellular domain cDNA constructs which code for the different amino acid combinations have been generated, and the protein variants expressed in COS-7 cells. Following antibody affinity purification, SDS-PAGE gel analysis revealed a single protein band of approximately 130KDa, which shifted to approximately 95KDa following N-deglycosylation. The variants were analysed by their ability to bind leptin via a radioactive ligand binding assay. Preliminary results establish that the protein variants are able to bind leptin, enabling future kinetic studies to determine potential differences between SNPs.

**Distinct cellular and biochemical effects of specific mutations in ABCA1 predicts the phenotype in TD and FHA.**

*R. Singaraja, H. Visscher, E. James, M. Hayden.* Dept Medical Genetics/CMMT, Univ British Columbia, Vancouver, BC, Canada.

We generated in vitro 15 missense mutations described in the ABCA1 gene, and characterized changes in HDL-C levels in patients harboring these variants. In order to elucidate the mechanism underlying the varying phenotypic effects caused by these mutations, specific functional assays were established exploring different stages of pathways of activation of ABCA1, including subcellular localization by immunofluorescence, glycosylation status and cell surface localization by biotinylation, ApoA-I binding and both phosphocholine and cholesterol efflux assays. These assays reveal a distinct correlation between the biochemical defects of the variants and disease severity in the patients with mutations and provide insights into the structure function relationships of ABCA1. Based on patient HDL-C levels, we hypothesized that in those homozygous for ABCA1 mutations, the patients with both alleles of ABCA1 showing no activity would have almost 0% HDL-C, whereas those with both alleles showing partial activity would have >10% HDL-C levels when compared to controls. Of the heterozygotes, those with the mutant allele retaining partial activity would be expected to show >50% HDL-C levels, those in whom the mutant allele lacks activity would be expected to show ~50% HDL-C levels, and those in whom the mutant allele acts in a dominant-negative manner would be expected to show <50% HDL-C levels. Indeed, when the HDL-C levels of all patients harboring specific mutations were compared to LRC age and sex matched controls, the patients could be thus classified. Importantly, there was a correlation between the different phenotypic groups and the in vitro biochemical activity of ABCA1. Taken together, our results show that discrete functional effects caused by changes in ABCA1 underlie the observed variation in disease severity and phenotype observed in TD and FHA patients.

**Fabry disease: Characterization of the D313Y plasma -galactosidase A pseudodeficiency allele.** *M. Yasuda*<sup>1,2</sup>, *J. Shabbeer*<sup>1</sup>, *S.D. Benson*<sup>3</sup>, *I. Maire*<sup>4</sup>, *R.M. Burnett*<sup>3</sup>, *R.J. Desnick*<sup>1</sup>. 1) Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY; 2) Dept Pediatrics, Tokyo Women's Medical University, Tokyo, Japan; 3) The Wistar Institute, Philadelphia, PA; 4) Laboratoire de Biochimie Pédiatrique, Hôpital Debrousse, Lyon, France.

Fabry disease, an X-linked inborn error of glycosphingolipid catabolism, results from mutations in the gene encoding the lysosomal exoglycohydrolase, -galactosidase A (-Gal A). In two unrelated classically affected males, two -Gal A missense mutations were identified: R112C + D313Y and C172G + D313Y. The D313Y lesion was previously identified in classically affected males as the single mutation [Eng et al., 1993] or in cis with another missense mutation, D313Y + G411D [Guffon et al., 1998]. To determine whether the D313Y mutation was a deleterious mutation, or a coding region sequence variant, the frequency of D313Y in normal X-chromosomes, as well as its enzymatic activity and subcellular localization in COS-7 cells were determined. D313Y occurred in 0.45% of 883 normal X-chromosomes, while the R112C, C172G, and G411D missense mutations were not detected in over 500 normal X-chromosomes. Expression of D313Y in COS-7 cells resulted in ~60% of wild-type enzymatic activity and showed lysosomal localization, while R112C, C172G, G411D, and the double-mutated constructs had markedly reduced or no detectable activity and were all retained in the endoplasmic reticulum. The expressed D313Y enzyme was stable at lysosomal pH (pH 4.6), while at neutral pH (pH 7.4), it had decreased activity. A molecular homology model of human -Gal A, based on the X-ray crystal structure of chicken -galactosidase B (-Gal B; -N-acetylgalactosaminidase) [Garman et al., 2002] was generated, which provided evidence that D313Y did not markedly disrupt the -Gal A enzyme structure. Thus, D313Y is a rare exonic variant with about 60% of wild-type activity in vitro and markedly reduced activity at neutral pH, resulting in low plasma -Gal A activity.

**Towards comprehensive characterization of the human retinal transcriptome.** *H.L. Schulz, H. Stöhr, J. Stojic, B.H.F. Weber.* Institute of Human Genetics, University of Wuerzburg, Germany.

The human retina is a complex tissue specialized in the conversion of light into neuronal signals which are then transmitted to the brain. An as yet unknown number of active genes are required to perform these versatile functions and to establish and maintain the structure of the individual retinal cell types. As a consequence, this highly evolved system is susceptible to a great number of genetic defects, thus leading to a wide range of retinal disease phenotypes. To date, more than 130 distinct genes have been linked to monogenic retinal dystrophies. Fewer advances have been made in dissecting the molecular basis of the multifactorial age-related macular degeneration, the most frequent cause of legal blindness in the developed world.

Our research, part of the German Human Genome Project, targets the identification and characterization of genes exclusively or preferentially expressed in the retina. To achieve this, two complementary approaches were used, namely the assessment of retinal expressed sequence tag (EST) clusters indexed in the UniGene database and the large scale generation of single-pass ESTs derived from a human retina suppression subtracted cDNA library. A total of 6616 EST clusters from both sources were evaluated and from these 879 were subjected to detailed in silico analysis. The expression profiling of 337 selected EST clusters was achieved by RT-PCR, conventional and virtual Northern blot, and real-time PCR. These methods led to the identification of 67 transcripts expressed abundantly or exclusively in retina and 48 genes expressed in retina and other areas of the neuronal system. To date, the full-length coding sequence of 47 genes has been established and the possible involvement of five genes in retinal diseases has been examined. In order to assess the completeness of the retinal transcriptome, the data assembled and analyzed in this project was compared with the reports of other retina-gene identification projects. The results indicate that there are still numerous retina-specific genes waiting to be discovered.

**Evaluation of Stat5b target genes in non-obese diabetic (NOD) mice by northern blot analysis and DNA microarray.** A. Davoodi-Semiromi<sup>1</sup>, S. Litterland<sup>1</sup>, M. Atkinson<sup>1</sup>, J-X. She<sup>2</sup>, M. Clare-Salzler<sup>1</sup>. 1) Dept of Pathology, Immunology and laboratory Medicine, PO Box 100275, Univ Florida, Gainesville, FL; 2) Center for Biotechnology and Genomic Medicine, Medical College of Georgia, 1120 15th Street, PV6B108, Augusta, GA 30912.

In human and in mice it has been generally accepted that type 1 diabetes is a multifactorial autoimmune disease in which susceptibility is determined by genetic and environmental factors. We have recently reported a natural novel point mutation in DNA binding domain of Stat5b in the NOD mice using mutation and functional assays. In mice, this gene is located on chromosome 11 where one of candidate interval for type 1 diabetes, *idd4*, has been mapped to this region but the gene yet to be identified. In this study, we assessed expression of putative Stat5b target genes, genes bear stat5b consensus sequence in their promoter region, by Northern blot analysis. The NOD mice (10 weeks) and normal strain (B6) were induced by GM-CSF (intraperitoneally injection) for 20 min and 2 hours and then total RNA were extracted from spleen and subjected to northern blot and microarrays for cytokines and their receptor and inflammatory genes. The northern blot data shown that the expression level of some of these genes has been changed in NOD mice when compared to normal strain. Using gene specific microarrays for cytokines and their receptors and inflammatory genes we not only confirmed the northern blot data, but also identified several genes in which their expression has been changed in the NOD mice. All of these genes bear the stat5b consensus sequence in their promoter region suggesting that their expression might be under the control of the Stat5b gene. Interestingly, two of these gene are just one and 2 cM away from the *Idd4* (chromosome 11) and *Idd13* (chromosome 4) loci, respectively. This study candidate two genes for the *Idd4* and *Idd13* in mice and more investigation is underway in order to rule in and/or rule out the candidacy of these genes in the pathogenesis of type one diabetes in mice. This study supported by an advance postdoctoral fellow ship (JDF10-2001-589) awarded to the ADS.

**Phosphorylation of the fragile X mental retardation protein regulates translation of its mRNA ligands.** *W.T. O'Donnell, S. Ceman, S.T. Warren.* Human Genetics, Emory Univ Sch Med, Atlanta, GA.

Fragile X mental retardation protein, FMRP, is absent in patients with fragile X syndrome, the most common inherited form of mental retardation. FMRP is an RNA binding protein that shuttles into and out of the nucleus and is found associated with polyribosomes. In neurons FMRP is found beneath dendritic spines and is thought to play a role in synapse maturation. FMRP is a translational repressor in cell free in vitro systems and may regulate the translation of its specific mRNA ligands in vivo. Using mass spectrometry we identify a fragment of FMRP that is phosphorylated in both murine brain and in cultured cells. By site-directed mutagenesis we show primary phosphorylation occurs on the highly conserved serine 499 residue. This first event then triggers hierarchical phosphorylation of at least one nearby serine. In contrast to the *Drosophila* ortholog dFxr, the phosphorylation status of mammalian FMRP neither affects its ability to dimerize nor influences its association with specific mRNAs in vivo. However, we show that a serine to alanine point mutant of FMRP mimicking dephosphorylation associates with actively translating polyribosomes while a serine to aspartate mutant mimicking phosphorylated FMRP associates with stalled polyribosomes. We analyze the specific messages associated with these translating or stalled polyribosomes by microarray and compare those sets of messages with the in vivo FMRP mRNA ligands. Of the 65 transcripts that co-immunoprecipitate with FMRP, 61 (94%) are found enriched greater than 4-fold in the heavy fractions of a linear sucrose gradient of azide treated cells containing Asp-FMRP compared to those from an azide treated Ala line. Our data suggest the phosphorylation status of mammalian FMRP regulates the translation of FMRP-associated mRNAs and dephosphorylation may be a trigger for translation during synapse formation.

**Variation of histone acetylation/methylation in the FMR1 gene of the fragile X syndrome following pharmacological reactivation.** *E. Tabolacci, R. Pietrobono, P. Chiurazzi, G. Neri.* Institute of Medical Genetics, Catholic University, Rome, Rome, Italy.

Fragile X syndrome is caused in most cases by a >200 CGG repeat expansion and methylation of the FMR1 gene, which, as a consequence, becomes inactive. We demonstrated that treatment of fragile X lymphoblasts with 5-azadeoxycytidine (5-azadC) reactivates the FMR1 gene (Hum Mol Genet 7:109-113, 1998), by demethylating the CpG residues of the promoter region (Nucl Acids Res 30: 3278-3285, 2002), and that histone hyperacetylating drugs synergistically potentiate the FMR1 gene reactivation induced by 5-azadC (Hum Mol Genet 8:2317-2323, 1999). We went on to investigate the histone acetylation and methylation status using ChIP assay with antiacetylated-H4/ -H3 antibodies and with anti-H3 dimethylated-K4/ -K9 antibodies (Upstate Biotechnology). To quantify the IP-DNA, fluorescent PCR (ABI 7700) was performed with three different TaqMan probes for the FMR1 and HPRT (internal control) genes, respectively. Here we provide evidence that 5-azadC treatment induces an increase of H3 and H4 acetylation and of K4-H3 methylation but a decrease of K9-H3 methylation, on three fragile X cell lines. Then we have explored the potential acetylating action of acetyl-L-carnitine (ALC), an FDA-approved drug, which reduces fragile sites expression in vitro (Am J Med Genet 51: 447-450, 1994). After ALC treatment, H3 and H4 acetylation levels undergo a change similar to that induced by 5-azadC; K9-H3 methylation decreases, while K4 methylation is only slightly increased. Finally, we have studied these histone modifications in an unmethylated full mutation cell line (5106): surprisingly, H3 and H4 acetylation and K9 methylation patterns are similar to those of a methylated full mutation, while K4 methylation resembles that of a wild type cell line, in accordance with its normal transcriptional activity. Supported by grants from FRAXA Foundation, Conquer Fragile X, Sigma-Tau, MIUR, Fondazione Cariplo and Associazione Anni Verdi, Italy.

**Evaluation of *Caenorhabditis elegans* model for lysosomal diseases caused by deficits of glycosidases. M.**

*Hrebicek, J. Sikora, J. Hujova, D. Holanova, H. Poupetova, B. Asfaw, J. Ledvinova, M. Kostrouchova.* 1st Faculty Med, Char, Institute of IEM, Praha 2, Czech Republic.

We have studied the relevance of *C.elegans* as a model for the study of molecular pathology of Pompe disease (-glucosidase - GAA), Fabry disease (-galactosidase, -GAL), and Schindler disease (-N-Acetyl-galactosaminidase, -NAGA). GAA, -GAL and -NAGA measurements in *C.elegans* mixed culture shown significant activities in the range of tens to hundreds of nmol/mg protein/hour for all three enzymes. BlastP searches for potential GAA, -GAL and -NAGA orthologs in the *C.elegans* genomic database, revealed four predicted genes with high GAA family homology (Ce GAA 1- 4) and a single predicted gene (Ce GAL/NAGA) both for -GAL and -NAGA. Phylogenetic analysis of evolutionary relevant sequences further supported common ancestral origin of mammalian -GAL and -NAGA. Full length cDNAs for Ce GAL/NAGA and Ce GAA 1 were amplified and sequenced including 5 and 3 UTR to confirm the in-silico prediction. Sequencing revealed SL1 trans-splicing RNA pattern for Ce GAL/NAGA and SL2 trans-splicing for Ce GAA 1. C-terminal GFP tagging was used to evaluate the expression pattern of the studied genes and to define their functional promoter. 5kb extrachromosomal GFP expressing promoter Ce GAA 1 construct showed broad tissue expression pattern including intestine, pharyngeal and vulval muscle, coelomocytes and head and tail neurones during all developmental stages of *C.elegans*. RNA interference (RNAi) method employing previously sequenced cDNA clones and genomic DNA as templates for dsRNA synthesis was performed to evaluate phenotypic presentation of Ce GAA 1 RNAi induced deficiency. Strong egg-laying phenotype was observed in at least 20% of the progeny of either N2 or rrf-3 injected worms. Translational C-terminal GFP construct of Ce GAL/NAGA showed coelomocyte vesicular compartment positivity after lysosomal alkalinisation with ammonium chloride or concanamycin.

**Molecular and biophysical characterization of Voltage-Dependent Anion Channel homologs in *Drosophila melanogaster*.** B.H. Graham<sup>1</sup>, A. Komarov<sup>2</sup>, M. Colombini<sup>2</sup>, W.J. Craigen<sup>1</sup>. 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Biology, University of Maryland, College Park, MD, 20742.

Voltage-dependent anion channels (VDACs) are a family of small pore-forming proteins of the mitochondrial outer membrane found in all eukaryotes. VDACs play an important role in the regulated flux of metabolites between the cytosolic and mitochondrial compartments and three distinct mammalian isoforms have been identified. The specific physiologic and potential pathophysiologic roles of the various isoforms are not understood. Recent annotation of the *Drosophila melanogaster* genome has revealed three putative VDAC isoforms (*CG17137*, *CG17139*, *CG17140*) closely linked to the previously reported *Drosophila* VDAC (*porin*). Molecular characterizations of these genes reveal a male-specific expression pattern for all three genes as well as potential dicistronic expression for *CG17139* and *CG17140*. When expressed in yeast deficient for VDAC, *porin* and *CG17137* rescue a conditional-lethal phenotype, demonstrating complementation. Electrophysiological characterizations of these proteins in reconstituted systems demonstrate a differential ability to permeabilize lipid bilayers and liposomes, with *CG17137* displaying the most prototypic VDAC behaviour. These studies indicate that *Drosophila* VDAC homologs have retained aspects of VDAC structure and function, warranting further studies to utilize this genetic model system in delineating fundamental conserved roles of VDACs in eukaryotic cell function.

**C5545T: a novel functionally dominant mitochondrial DNA mutation.** *M. Davidson, S. Sacconi, L. Salviati, E. Hernandez, Y. Nishigaki, R. Pons, D. DeVivo, S. Shanske, E.A. Schon, E. Bonilla, S. DiMauro.* Columbia University, New York, NY.

Several pathogenic mitochondrial tRNA mutations have been described in patients with mitochondrial diseases. The wild type and mutant molecules coexist (heteroplasmy) within a cell. Generally, clinical symptoms of the disease are manifested only when the percentage of mutant molecules exceeds 70-90% indicating that these mutations are functionally recessive. We studied a 11-year old patient who presented with brain, heart and muscle involvement. Muscle histochemistry revealed a generalized reduction in COX histochemistry and no ragged-red fibers, unlike other tRNA mutations. Analysis of respiratory chain enzymes showed 88% reduction in complex IV and 25-50% reduction in other complex I and III. Sequence analysis of the tRNA genes revealed a C->T transition at nt5545 in tRNA<sup>Try</sup> gene, which alters the anticodon sequence (UGA) to a stop codon (UAA). The mutation was present at unusually low level in patients muscle (around 25%) and at even lower levels in patients blood and fibroblasts (13-17%), while it was absent in the mothers tissues. Contrary to other pathogenic tRNA mutations, where COX-negative fibers harbor more than 90% mutated mtDNA, single fiber analysis of the patients muscle biopsy revealed a 31% mutation load in COX-negative fibers, while the mutation was virtually absent in COX-positive fibers. We have ruled out the presence of a secondary mtDNA mutation in the other tRNA genes and COX subunits by sequencing. Screening for SCO1, SCO2 and SURF1 nuclear genes were negative.

This is the first mtDNA mutation to affect the central base of the anticodon in a tRNA gene. The pathological threshold in muscle was around 30% or less. Despite the low mutation load, the patient showed a severe biochemical and clinical phenotype. The functional dominance of this mutation is the exception to the general rule and therefore, the pathogenic mechanism may be different from other mutations studied. There may be other dominant mtDNA mutations that may have been overlooked because of the limit of detection by conventional methods.

**Ablation of Mitochondrial Intermediate Peptidase gene leads to embryonic lethality during mouse gastrulation.**

*R.T. Mantcheva*<sup>1</sup>, *A. Chew*<sup>2</sup>, *A.R. Thornhill*<sup>3</sup>, *J.M. van Deursen*<sup>1</sup>, *G. Isaya*<sup>1</sup>. 1) Dept. of Pediatric & Adolescent Medicine and Biochemistry & Molecular Biology, Mayo Clinic, Rochester, MN; 2) Genaisance Pharmaceuticals, Inc., Five Science Park, New Haven, CT; 3) Dept. of Obstetrics & Gynecology, Mayo Clinic, Rochester, MN.

Most mitochondrial precursor proteins are processed to the mature form in one step by mitochondrial processing peptidase (MPP), while a subset of precursors destined for the matrix or the inner membrane are cleaved sequentially by MPP and mitochondrial intermediate peptidase (MIP). MIP cleavage sites are found in respiratory components, including subunits of the electron transport chain, tricarboxylic acid cycle enzymes, iron-sulfur cluster-containing proteins and ferrochelatase, as well as components of the mitochondrial genetic machinery, including ribosomal proteins, translation factors, and proteins required for mitochondrial DNA metabolism. MIP catalyzes the maturation of ferrochelatase. We showed previously that yeast MIP (YMIP) is required for mitochondrial function in *Saccharomyces cerevisiae*. To further define the role played by two-step processing in mitochondrial biogenesis, we have generated a mouse model by deletion of exons 12 and 13. The deletion encompasses the peptidase active site and is predicted to result in the inactivation of the MIP gene product. We show that homozygosity for this deletion causes embryonic lethality a few days after implantation, demonstrating an important role for MIP during early development. Consistent with the fact that some of the MIP substrates are essential for the maintenance of oxidative phosphorylation (OXPHOS), these results suggest that high energy demands during early development are not met in the absence of MIP due to impaired proteolytic processing.

Program Nr: 1048 from 2003 ASHG Annual Meeting

**The Induced Mutant Resource: Mouse mutants with applications to human disease.** *S.F. Rockwood, D.B. Lane, L.E. Mobraaten, M.T. Davisson.* Induced Mutant Resource, Jackson Laboratory, Bar Harbor, ME.

Advances in transgenic and gene targeting technologies have yielded a wide variety of mouse models that have proven to be extremely useful in addressing questions that explore the mechanisms of human disease. To ensure the greatest possible access to these tools, the Induced Mutant Resource (IMR) at The Jackson Laboratory was created in 1992 to serve as a centralized facility to collect, cryopreserve and distribute induced mutant mice to the scientific community. Since its inception, the IMR has accepted over 950 different induced mutant mouse strains, distributing over 130,000 mice annually to the scientific community. Current growth in the IMR collection is approximately 60-70 new strains each year.

A wide variety of new mutants have recently been accepted into the IMR. A targeted mutant of the *Aire* (autoimmune regulator) gene, useful for studies related autoimmune disease is currently under development. Also new, are models for fragile X mental retardation syndrome and Huntington's disease. Floxed alleles of the *Pten* (phosphatase and tensin homolog) and *Bdnf* (brain derived neurotrophic factor) genes have been added to the growing list of loxP-flanked alleles. One of the more creative additions to the IMR is a diphtheria toxin-inducible transgenic mutant that allows scientists to transiently deplete dendritic cells.

An on-line resource is available allowing researchers to retrieve information related to the mutant strains maintained in the IMR. Mutant strain records retrieved from the IMR database include brief phenotype descriptions, strain construction and husbandry information and a listing of related references. Researchers wishing to have mutant strains considered for inclusion in the IMR collection may submit their strains using the form available at the IMR web site: <http://www.jax.org/imr/index.html>.

The Induced Mutant Resource is supported by the National Center for Research Resources (RR09781/RR11081), National Institute for Allergy and Infectious Disease and The Howard Hughes Medical Institute.

**Identification and characterization of a DNase hypersensitive region of the human tyrosinase gene.** *W.S. Oetting<sup>1,2</sup>, J.P. Fryer<sup>1</sup>, R.A. King<sup>1,2</sup>*. 1) Dept. Medicine; 2) Institute of Human Genetics, Univ Minnesota, Minneapolis, MN.

A significant number of individuals with oculocutaneous albinism (OCA) have been found to have only one heterozygous mutation of the tyrosinase gene, when the analysis includes the entire coding region, flanking intron/exon boundaries, and 500 bp of the proximal promoter. Based on the allele frequency of mutant tyrosinase alleles, we assume that these individuals have OCA1, rather than a type of OCA resulting from mutations of another locus while also being carriers of a tyrosinase mutation. Our inability to detect the second tyrosinase mutation suggests that these cryptic mutations in the homologous allele could reside in regulatory regions that are removed from the direct proximity of the coding sequence, and this would account for their having OCA1. It has been previously reported that the mouse tyrosinase gene has a distal enhancer that provides position-independent stimulation of tyrosinase gene expression. A sequence homology search between the mouse and human tyrosinase promoter has identified a region of the human tyrosinase promoter that is similar to this mouse distal enhancer. We report a region of the human tyrosinase promoter, 9 kb upstream of the transcriptional start site, which exhibits DNase I hypersensitivity in a cell lineage-specific manner. This region also has significant enhancer function when reporter vectors constructs containing this region are transfected into either human or mouse melanocyte cell lines. Furthermore, elimination of core sequences extinguishes the enhancer function. We believe that this region contains sequences critical in the regulation of the human tyrosinase gene and is a candidate for the location of cryptic mutations in OCA1.

**The Effect of Sarcoglycan Mutations on the Assembly of the Sarcoglycan Complex.** *Y. Chan, W. Shi.* The Henry Hood Research Program, Sigfried and Janet Weis Center for Research, The Geisinger Clinic, Danville, PA 17822.

Sarcoglycans are a group of novel single-pass transmembrane proteins predominantly expressed in skeletal and cardiac muscle. They form a specific multi-meric complex on the muscle membrane. Mutations in sarcoglycans have been reported to cause the autosomal recessive Limb-girdle muscular dystrophy (LGMD) type 2C-F as well as dilated cardiomyopathy (DCM). Using a heterologous expression system, our previous study has demonstrated that the assembly of the sarcoglycan complex occurs at a discrete step-wise process and involves the formation of a  $\alpha$ -sarcoglycan core structure. In this report, we use the same system to examine the effect of sarcoglycan mutations on the sarcoglycan complex. Our data have shown that different mutations perturb specific stages in the assembly process of the sarcoglycan complex.  $\alpha$ -sarcoglycan mutants defective in glycosylation result in aggregation and disrupt its interaction with  $\beta$ -sarcoglycan. Disease-causing point mutations near the N-linked glycosylation sites of sarcoglycans have an adverse effect on glycosylation. A common mutation in  $\beta$ -SG (C283Y) is shown to affect its association with the  $\alpha$ -sarcoglycan core structure. Interestingly, a mutation in the intracellular domain of  $\beta$ -sarcoglycan (Q11E) has no effect on sarcoglycan complex formation. This raises the possibility that some mutations in sarcoglycans might affect a function unrelated to the assembly process. Understanding the molecular mechanism on how a particular sarcoglycan mutation causes LGMD or DCM will likely provide specific strategy for disease treatment in the future. This information might also benefit clinicians in disease management by better addressing the phenotypic variation in LGMD.

**EFFECT OF FILAMINC DEFICIENCY IN MUSCLE.** *I. Dalkilic<sup>1</sup>, L.M. Kunkel<sup>1,2</sup>*. 1) Dept Genetics, Children's Hospital, Boston, MA; 2) Howard Hughes Medical Institute.

Filamins (A, B, and C), are a family of actin binding proteins. FilaminC (FLNC) is muscle specific, expressed in heart and skeletal. FLNC previously has been shown to interact with d- and g- sarcoglycans, which when mutated give rise to LGMD2F and 2C. FLNC is localized to the Z-disc and at very low levels to the sarcolemma (~3%). Interestingly in DMD and LGMD2C where the sarcoglycan complex is lost from the membrane the amount of FLNC at the membrane increases (~24%). Apart from the sarcoglycans, FLNC has been shown to interact with other proteins involved in muscular dystrophies, such as myotilin and calpain-3. All of this evidence indicates an important role for FLNC in muscle and muscular dystrophy pathogenesis, although to date no disease has been found to have mutations in the FLNC gene. To study the effects of FLNC loss in the skeletal muscle we have taken two different approaches. First we have created a partial knockout model that deletes the portion of FLNC shown to interact with the sarcoglycans and calpain-3. This knockout is embryonic lethal. We are currently investigating these knockouts further to deduce the reason for embryonic lethality. The second approach is the use of siRNAs (small interfering RNAs) to knockdown the expression of FLNC in mouse myoblast cell lines (C2C12). siRNAs are 19-23 nt long double stranded RNAs, which through their homology target the endogenous mRNA for degradation, hence knocking down the expression. We have applied this system to knockdown the expression of FLNC in C2C12 cell line and achieved 80% decrease in FLNC expression with various siRNAs. We are currently investigating the phenotype of cells with FLNC deficiency.

**Retinal dystrophin isoform Dp260 promoter includes a GRE (glucocorticoid response element) and transcription is upregulated *in vitro* by steroids.** *D.M. Pillers*<sup>1,2</sup>, *J.T. den Dunnen*<sup>3</sup>, *J. Pang*<sup>1</sup>. 1) Dept Pediatrics, Oregon Health & Science Univ, Portland; 2) Dept Molecular and Medical Genetics, Oregon Health & Science Univ, Portland; 3) Human and Clinical Genetics, LUMC, Leiden, Nederland.

Introduction: Dystrophin is the product of the Duchenne muscular dystrophy gene (DMD). In addition to full length dystrophin (Dp427), the DMD gene encodes several shorter isoforms that vary in their tissue distribution. The redundancy in the composition of the isoforms offers the potential to substitute for Dp427 to ameliorate the Duchenne muscular dystrophy phenotype. We have investigated the potential of pharmacogenetic manipulation as a means of upregulating the expression of specific isoforms. Methods: A human genomic fragment from a cosmid clone containing the first exon of Dp260 was characterized, and a fragment was subcloned in a pGL2 luciferase reporter vector that was transfected into cell lines Y79 (retinoblastoma), HeLa, COS-7, and C2 (myoblast) for assays of transcription and pharmacologic intervention. Results: Sequence analysis using the TESS (Transcription Element Search System) identified a glucocorticoid response element (GRE). Exposure of transfected cell lines to methylprednisolone resulted in upregulation of activity as demonstrated by relative luciferase activity: Y79 (2 fold), HeLa (6 fold) and C2 (6 fold). COS-7 transfectants showed no effect after steroid exposure, as expected for this GR defective cell line. Conclusions: The retinal dystrophin Dp260 promoter includes a GRE which when exposed to steroids *in vitro* leads to upregulation of Dp260 transcription in a variety of cell lines, including muscle-derived (C2). In clinical studies, steroidal agents have been associated with improvement in muscle disease in some individuals. Our results indicate that pharmacogenomic approaches to DMD gene product expression may be useful as potential therapies for muscular dystrophy.

**Mutation and single nucleotide polymorphisms in *RUNX2* and *CBFb* genes in patients with cleidocranial dysplasia (CCD).** *D. Napierala*<sup>1</sup>, *X. Garcia-Rojas*<sup>1</sup>, *R. Mendoza*<sup>2</sup>, *Q. Zheng*<sup>2</sup>, *G. Zhou*<sup>2</sup>, *E. Munivez*<sup>1</sup>, *Y. Chen*<sup>2</sup>, *B. Lee*<sup>1,2</sup>. 1) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX; 2) Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston TX.

Cleidocranial dysplasia (CCD) is a dominantly inherited condition characterized by hypoplastic clavicles, large fontanel, dental abnormalities and delayed skeletal development. It is caused by mutations in *RUNX2*, a transcription factor essential for osteoblasts differentiation. The majority of mutations are missense and abolish *RUNX2* binding to a target OSE2 sequence. These and additional nonsense, splicing and frame shift mutations are detectable in approximately 60% of CCD patients. It has been recently described that mice carrying a *CBFb* hypomorphic allele have a skeletal phenotype similar to CCD. *Cbfb* acts as a transcriptional coactivator with runt domain proteins. The objective of the current study was to describe additional mutations leading to CCD phenotypic spectrum. We performed PCR amplification of genomic DNA for *RUNX2* gene and sequenced all of its 8 exons for 14 unrelated patients. For 27 CCD patients with no mutation identified in *RUNX2* coding region, we performed sequencing analysis of a 1.3 kb fragment of *RUNX2* promoter and all of the 6 exons of the *CBFb* gene. A total of 3 new mutations in *RUNX2* coding region, 4 variants in the *RUNX2* promoter and 3 variants in *CBFb* gene were detected. We analyzed the frequency of all detected SNPs in general population. DHPLC analysis in 180 control chromosomes showed two common polymorphisms in *RUNX2* promoter with allele frequencies of 10% and 15%. Two other sequence variants were not found in the control group. Both rare sequence variants occur within zinc finger transcription factors binding sites. Electrophoretic mobility shift assay (EMSA) using nuclear extracts from ROS17, 10T1/2 cell lines showed a specific protein-DNA complex corresponding to both cis elements. These data suggest that mutations in *CBFb* do not contribute to the CCD spectrum. Additionally, analysis of the CCD cohort has identified SNPs in the *RUNX2* promoter that may be relevant cis-elements for transcriptional regulation of *RUNX2* and the pathogenesis of CCD.

Program Nr: 1054 from 2003 ASHG Annual Meeting

**The human PRKCQ gene is positively associated with rheumatoid arthritis.** *S. Hall, C. Stack, K. Magnuson, A. Seymour.* Pfizer, Groton, CT.

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases. It is a chronic disease characterized by prolonged inflammation, swelling and pain of multiple joints. Activated T-cells are thought to play a role in initiating the disease as well as driving the chronic inflammatory process. PKC theta demonstrates restricted expression in T lymphocytes and skeletal muscle and has been shown to be required for T cell activation. In this study, we have investigated whether genetic polymorphisms with the PRKCQ gene are associated with an increased susceptibility to RA.

A case control genetic association study design was used to test the hypothesis that polymorphisms within the PRKCQ gene contribute to disease susceptibility. 120 RA subjects and 120 age, ethnic, and CRP/RF control subjects were genotyped for 11 polymorphisms evenly spaced across the 87 kb PRKCQ gene. Logistic regression models were used to compare the relationship between PRKCQ genotype and RA phenotype. Several polymorphisms were found to be highly significant with p-values ranging from 0.02-0.05. We identified a haplotype that is associated (p-value = 0.005) and represents an odds ratio of 1.7, assuming a dominant model of inheritance. We conclude that polymorphisms in the PRKCQ gene are associated with an increased susceptibility to RA.

**Abnormal connective tissue remodeling in mice deficient for relaxin receptor (LGR7).** A.A. Kamat, S. Feng, N. Bogatcheva, A.I. Agoulnik. Obstetrics & Gynecology, Baylor College of Medicine, Houston, TX.

Relaxins are peptide hormones produced primarily in reproductive organs. In the mouse, there are two relaxin genes, Rln1 and Rln3 that encode related peptides with an overlapping pattern of expression. Deletion of relaxin-1 in mice causes remodeling of connective tissues in several organs. Recently, biochemical studies have identified two closely related G protein-coupled receptors (LGR7 and LGR8/GREAT) as putative receptors for relaxins. Additionally, insulin-like factor 3 has been identified as a cognate ligand for Lgr8, but not for Lgr7. Using real time polymerase chain reaction, we have shown that the Lgr7 receptor is expressed in a variety of murine organs including reproductive organs, brain, heart, aorta, etc. Using insertional gene targeting approach, we targeted the lacZ gene into the mouse Lgr7 locus.

Lgr7 deficient mice have abnormal mammary development with smaller, poorly developed nipples. Mutant females are unable to deliver milk to their pups. The uterine size appears to be smaller in mutant mice without any alteration in morphology. These females also have significantly prolonged length of parturition compared to littermates. Examination of sections of the lung in Lgr7 deficient mice reveals pulmonary fibrosis that is seen as early as one month of age compared to 6 months in relaxin-1 knockout mice. Additionally, there is excessive deposition of connective tissue around pulmonary veins, which is not seen in controls. Examination of collagen content using trichrome staining of histological sections reveals increased collagen deposition around pulmonary veins in mutant mice. Thus, analysis of the Lgr7 deficient mice reveals a more severe phenotype than the one in relaxin-1 deficient mice, indicating ligand redundancy for Lgr7 in vivo.

We have also produced mice with a deficiency of both Lgr7 and Lgr8 receptors, as well as Lgr7 deficient mice with an additional transgenic copy of Insl3. Analysis of their phenotype did not reveal additional phenotypic abnormalities, indicating a non-overlapping nature of the relaxin and Insl3 signaling in vivo.

**Polymorphisms and functional analysis in the promoter of the interleukin-4 receptor alpha chain gene.** *N.*

*Hosomi<sup>1</sup>, K. Fukai<sup>1</sup>, N. Oiso<sup>1</sup>, A. Kato<sup>1</sup>, T. Murakami<sup>1</sup>, M. Ishii<sup>1</sup>, H. Kunimoto<sup>2</sup>, K. Nakajima<sup>2</sup>.* 1) Department of Dermatology, Osaka City University Graduate School of Medicine, Osaka, Japan; 2) Department of Immunology, Osaka City University Graduate School of Medicine, Osaka, Japan.

The gene encoding the interleukin-4 receptor alpha chain (IL4RA) is a candidate gene for atopic diseases. Previously, we reported four polymorphisms in the IL4RA transcriptional promoter: -327A/C, -326C/A, -186A/G, -184A/G. These polymorphisms were mainly classified into two haplotypes: alpha (.ac..a.a..) and beta (.ca..g.a..), and alpha haplotype is more frequent in atopic dermatitis, and beta haplotype is more frequent in psoriasis as compared with healthy controls.

In this study, we further investigated -482 to -3249 of the 5-flanking region of the IL4RA and identified two novel polymorphisms: -1803T/C, -3112C/T and we carried out association study of these polymorphisms. In atopic dermatitis patients, at -1803T/C and -3112C/T, C allele and T allele are significantly higher (P=0.0034 and P=0.0075) than normal controls. Linkage disequilibrium between alleles at the SNP loci in the promoter and coding region of IL4RA was calculated. There was strong linkage disequilibrium between four SNPs, -327A/C, -1803T/C, -3112C/T and I50V.

We also investigated the functional analysis of the IL4RA transcriptional promoter in Jurkat and HaCaT cells. However, luciferase reporter assay revealed no statistical difference of the promoter activity between alpha haplotype and beta haplotype, and allele C and T at both -1803 and -3112. Although the computer-aided transcription search predicted that GATA-3, the Th2 type transcription factor, should bind only to -3112T allele of the promoter, co-transfection of GATA-3 expression vector did not up-regulate the promoter activity of our luciferase reporter system.

Despite the association of the six promoter polymorphisms with atopic dermatitis and psoriasis, *in vitro* transient expression analysis did not show evidence that these are functionally significant.

**Multi-species comparative sequence analysis of the *WS4/CMT1/Sox10* locus.** A. Antonellis<sup>1,4</sup>, S.Q. Lee-Lin<sup>1</sup>, L.L. Baxter<sup>2</sup>, T.G. Wolfsberg<sup>1</sup>, N.C.S. Program<sup>3</sup>, W.J. Pavan<sup>2</sup>, E.D. Green<sup>1,3</sup>. 1) Genome Technology Branch, NHGRI, NIH, Bethesda, MD; 2) Genetic Disease Research Branch, NHGRI, NIH, Bethesda, MD; 3) NIH Intramural Sequencing Center, NHGRI, NIH, Gaithersburg, MD; 4) Genetics Program, George Washington University, Washington, DC.

Mutations in the gene for the high-mobility group transcription factor *Sox10* have been implicated in Waardenburg-Shah syndrome (WS4) and a Charcot-Marie-Tooth disease type 1 (CMT1) phenotype. While the role of this gene is well documented in both neural crest cell development and myelin-specific gene regulation, the factors that regulate *Sox10* expression remain unclear. Indeed, there is currently no defined functional promoter at this locus. To understand the transcriptional regulation of *Sox10*, we have performed multi-species comparative sequence analysis, a powerful approach for identifying cis-acting transcriptional regulatory elements. For this analysis, nearly 150 kb of genomic DNA spanning the *Sox10* coding sequence plus upstream and downstream regions were sequenced in cow, pig, cat, dog, and rat, and added to the data already available for human and mouse. In addition, we have developed a highly stringent method for identifying short exact matches between orthologous sequences. By applying this method to the genomic sequence of these seven species and incorporating data from promoter and transcription factor binding site predictions, we have identified a number of putative biologically interesting regulatory elements and a potential gene promoter. We believe these results will facilitate two specific areas of research at the *Sox10* locus: (1) the identification of functional cis-acting regulatory elements, and (2) additional screening for disease-associated mutations in conserved non-coding sequences.

**The role of Steroidogenic Factor 1 (SF1) in transcriptional regulation of human *CYP17*.** A.S. Barbosa, C.J. Lin.  
Div. Endocrinol, Dep. Int. Med, University of Sao Paulo, Sao Paulo, Sao Paulo, Brazil.

The *CYP17* gene encodes cytochrome P450c17, a key enzyme for the production of corticosteroids and sex steroids. *CYP17* gene expression is regulated in a tissue-specific and species-specific fashion by trophic hormones. Human adrenals and gonads also exhibit basal transcription of *CYP17*. The promoter of human *CYP17* harbors four recognition sites for SF1, two NF-1 sites, one Sp1/Sp3 site, and a GATA site. To investigate the contribution of SF1 and GATA to basal *CYP17* transcription, mutant constructs containing isolated or combined inactivation of the SF1 and GATA sites were cloned into luciferase reporter vector and expressed in human adrenal NCI-H295A cells, which express SF1 endogenously, and COS-7 cells. Promoter activity was tested in the context of endogenous and overexpressed SF1. In SF1-overexpressing NCI cells, isolated inactivation of the SF1 sites decreased promoter activity by 75% (SF1-1mut), 50% (SF1-2mut), 37.5% (SF1-3mut), and 12.5% (SF1-4mut). The impact of these mutations is less prominent in the absence of SF-1 expression vector (respectively, 62, 31, 34 and 8,5%). In NCI cells, mutation of GATA site increased transcription in 12.5% when SF1 was overexpressed. Combined inactivation of SF1-1 and GATA sites, of the four SF1 sites, and of three SF1 sites (SF1-2/3/4mut) reduced activity by 70-87.5% when SF1 was overexpressed, but had little impact in the context of endogenously expressed SF1. In COS-7, isolated inactivation of any SF1 site decreased promoter activity by 50%, irrespective to its distance to the *TATA box*. GATA site inactivation increased activity by 50%, and combined inactivation of multiple SF1 sites reduced transcription by 75%. Our data indicate that: i) all four SF1 sites contribute to *CYP17* transcription, and, in adrenocortical cells, the influence of a SF1 site decreases as its distance to *TATA box* increases; ii) the GATA sequence might be part of an inhibitory recognition site; iii) the impact of overexpression of SF-1 in the activities of promoter/reporter constructs in NCI cells suggests that co-transfection of SF-1 expression vector may not recapitulate the physiological environment of adrenocortical cells.

Program Nr: 1059 from 2003 ASHG Annual Meeting

**Regulation of ADAM33 and Airway Remodeling.** *R. Del Mastro, H. Giese, R. Little, P. Van Eerdewegh.* Human Genetics, Genome Therapeutics Corp, Waltham, MA.

We previously identified ADAM33 as an asthma susceptibility gene using a positional cloning approach. Statistical analysis revealed disease-associated SNPs within the coding and noncoding regions of the gene. We hypothesize that the associated SNPs are within regions of the gene that affect the efficiency of sequence elements to recruit proteins necessary for transcriptional regulation and/or splicing modulation. We have performed a comprehensive search within ADAM33 using multiple computational analyses to identify regions that contain potential transcriptional regulatory and splicing elements (TRASE). We identified conserved non-coding sequences using the human and mouse ADAM33 genes within introns AB, BC and ST. The latter two introns contained conserved non-coding sequences that were within the vicinity of the most significant disease-associated SNPs. Exonic splicing enhancer elements (ESEs) were also identified within the gene and several of these cis-acting elements spanned significant disease-associated SNPs. The predicted TRASE and SNP data were integrated onto the ADAM33 gene structure. Using this map, we have generated a comprehensive set of mini-gene constructs to examine the effects of nine of the most significant SNP pair combinations (p-value below 0.0005) on transcription and splicing. Preliminary data suggest that these SNPs may have an effect on splicing modulation. Based on expression patterns, we have previously suggested that ADAM33 may play a role in airway remodeling. To gain an insight into ADAM33s function, we have designed a cell-based airway remodeling assay and coupled the RNAi technology to knockdown the gene. Using primary lung fibroblasts and bronchial smooth muscle cells, ADAM33 expression was reduced to 6% and 20% respectively. We were able to maintain these low levels of expression over 7 days post siRNA transfection. We have measured the effects of the ADAM33 knockdown in these primary cells by monitoring the expression of genes known to be involved in airway remodeling. The results from the use of this targeted cell-based assay and the evaluation of the mechanistic role of the SNPs on ADAM33 regulation will provide a greater understanding of the genetic factors that affect susceptibility to asthma.

**Functional characterisation of transcription factors deleted in Williams-Beuren Syndrome.** *P.D. Cunliffe, N. Hart-Holden, T. Hinsley, A.P. Read, M. Tassabehji.* Medical Genetics, University of Manchester, St Mary's Hospital, Manchester, UK.

Williams-Beuren syndrome (WBS) is a developmental disorder caused by deletion of approximately 1.5Mb on human chromosome 7q11.23 which occurs in approximately 1/20000 live births. Phenotypic features of the disease include the heart defect, supra-valvular aortic stenosis (SVAS), along with a distinctive cognitive profile combining mild mental retardation with an outgoing personality. Other phenotypic features include a dysmorphic face, growth retardation and hypercalcaemia. The only aspect of the WBS disease phenotype unambiguously associated with deletion of a particular gene is the SVAS, caused by deletion of the elastin gene. Detailed mapping of the WBS critical region has identified genes encoding a family of transcription factors as strong candidates for other aspects of the disease phenotype. The members of the family show a high degree of similarity to one another and contain varying numbers of a repeated motif known as an I repeat. Here we present functional data showing the effects of three of these genes (*GTF2I*, *GTF2IRD1* and *GTF2IRD2*) on downstream target genes. *In vitro* DNA binding studies show that *GTF2IRD1* binds a consensus DNA sequence present in several distinct enhancer elements via the fourth of its five I repeats. Luciferase assays show that both *GTF2IRD2* and some, but not all, isoforms of *GTF2IRD1* act as repressors of transcription from the *c-fos* promoter whereas *GTF2I* acts as an activator of this gene. Aberrant levels of protein from members of this family of genes are likely to influence expression of a number of downstream target genes that could result in some of the developmental abnormalities manifest in WBS.

Program Nr: 1061 from 2003 ASHG Annual Meeting

**Functional analysis of the transcriptional regulation of the human bile salt export pump gene.** *J. Byrne, S. Strautnieks, G. Mieli-Vergani, R. Thompson.* Institute of Liver Studies, King's College Hospital, Guy's, King's and St Thomas' School of Medicine, Bessemer Road, London, UK.

The bile salt export pump, BSEP, encoded by *ABCB11*, is the major bile salt transporter of human liver. *ABCB11* is mutated in a form of low <g>-GT progressive familial intrahepatic cholestasis (PFIC), known as BSEP deficiency. It has been documented that transcription of *ABCB11* is positively regulated by bile salts. However, little is known about which other transcription factors are involved in controlling the expression of the human gene, as well as the effect of exogenous compounds on its transcription. This study was designed to investigate these questions. Three *ABCB11* transcription start sites were identified using 5' Rapid Amplification of cDNA Ends (RACE) experiments, two of which are novel. These transcription start sites were found to be utilised both in normal liver and in the liver from patients with various cholestatic syndromes. 3' RACE experiments identified the previously unknown transcription termination site for the *ABCB11* mRNA. 2 kb of the promoter region of the human gene was subsequently amplified by polymerase chain reaction and cloned into the luciferase expression vector, pGL3-basic. 0.2 kb 5' deletion constructs were subsequently generated and cloned. All constructs were transfected into HepG2 cells and left for 48 hours before harvest. Analysis of the luciferase activity given for each deletion construct has identified areas of transcriptional importance in the human promoter, including a putative silencer motif. Current work is focusing on defining the exact role of these regions through the use of electrophoretic mobility shift assays. Furthermore, the ability of drugs and hormones to repress *ABCB11* transcription is being assessed. In conclusion, this work is providing an insight into the transcriptional regulation of *ABCB11* under normal and cholestatic conditions. Furthermore, it provides an experimental system to assess the effect of *ABCB11* promoter sequence variants found in cholestatic patients.

Program Nr: 1062 from 2003 ASHG Annual Meeting

**The Role of TFII-I and HDAC3 in  $\gamma$ -globin gene expression.** *V.J. Crusselle, K.F. Vieira, J. Bungert.* Biochemistry and Molecular Biology, University of Florida, Gainesville, FL.

High level and developmental stage-specific erythroid  $\gamma$ -globin gene expression is controlled by proximal and distal elements. It is well known that  $\gamma$ -globin gene expression is regulated by multiple transcription factors. We previously found that TFII-I, a helix-loop-helix protein, binds to the promoter region of the  $\gamma$ -globin gene in human embryonic erythroid cells in which the gene is repressed. Studies have shown that histone deacetylase 3 (HDAC3) is known to functionally and physically interact with TFII-I in COS7 cells. By deacetylating histones, HDAC3 preserves their basic nature and impedes DNA accessibility. We therefore hypothesized that TFII-I recruits HDAC3 to the promoter region of the  $\gamma$ -globin gene, thus inhibiting the expression of the  $\gamma$ -globin gene. ChIP and co-immunoprecipitation experiments showed that TFII-I and HDAC3 interact at the inactive  $\gamma$ -globin promoter in human embryonic erythroid cells. We then used RNA interference to knock down the expression of these proteins in murine adult erythroid cells and human embryonic erythroid cells and determined the effect on  $\gamma$ -globin gene expression. Our results suggest that TFII-I plays a role in the regulation of the  $\gamma$ -globin locus.

**Down-regulation of p21 gene expression by the transcription factor NF1.** *S. Ouellet<sup>1</sup>, S. Leclerc<sup>2</sup>, R. Drouin<sup>1</sup>, S. Guérin<sup>2</sup>.* 1) Division of Pathology, Departement of Medical Biology, Faculty of Medicine, Laval University and Unité de recherche en génétique humaine et moléculaire, Research Centre, Hôpital St-François d'Assise, Centre Hospitalier Universitaire de Québec, Canada; 2) Oncology and Molecular Endocrinology Research Center, CHUL Research Center (Québec), Canada.

P21waf1cip1 is known to mediate cell-cycle arrest, apoptosis, senescence and differentiation via specific protein-protein interactions with the cyclins, cyclin-dependent kinase (Cdk), PCNA, Topoisomerase II, etc. The regulation of p21waf1cip1 gene expression occurs mainly at the transcription level. Many DNA damaging agents strongly activate transcription of the p21waf1cip1 gene via a p53-dependent or independent mode, leading to cell-cycle arrest or apoptosis. P21waf1cip1 was also up-regulated in the apparent absence of genotoxic stress, following growth factors exposition, hormones or serum stimulation and for terminally differentiated cells. However, the cell proliferative influence of p21waf1cip1 appears to rely on the nuclear level of p21. Through the use of the Ligation Mediated PCR (LMPCR), we identified several DNA target sites on the proximal promoter of the human p21 gene for nuclear transcription factors expressed in primary cultured human fibroblasts. One of these sites contain a perfect consensus binding sequence for E2F1 whereas another bears a perfect consensus sequence for the members of the nuclear factor-1 (NF1) family of transcription factors. The role of the E2F transcription factor family in the transcriptional regulation of the p21 gene has already been reported previously but so far, nothing was known about the regulatory influence exerted by NF1 on the p21waf1cip1 transcriptional regulation. Using both EMSA and supershift analyses, NF1 was recognized as the major protein interacting with the most proximal footprinted region from the p21waf1cip1 promoter. Through transient transfections of recombinant constructs bearing the CAT reporter gene under the control of either the wild type p21 proximal promoter or a derivative in which the NF1 site was deleted, we demonstrated that NF1 can act as a repressor in primary cultured fibroblasts, human HeLa and rat pituitary GH4C1 cells. Our results therefore suggest a role for NF1 in the cell-cycle dependent expression of p21 both in vivo and in vitro.

**HNF4 is Essential for Expression of the Human Hepatic Lipase Gene (*LIPC*) in vivo: HNF4 and ARP-1 Bind to DR1 and DR4 Elements of the Promoter and Fine-Tune its Activity in vitro.** *B. Kurdi-Haidar*<sup>1</sup>, *S.A. Duncan*<sup>2</sup>, *M.A. Battle*<sup>2</sup>, *S.S. Deeb*<sup>1</sup>. 1) Departments of Medicine and Genome Sciences, University of Washington, Seattle, WA; 2) Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI.

The *LIPC* promoter contains two conserved direct repeats of the core motif RGG/TTCA with one- and four-base spacing (DR1 and DR4). HNF4a and ARP-1 together activate the *LIPC* promoter via the DR1 element, and excess ARP-1 suppresses this activation via the DR4 element. RARa/RXRa heterodimers, in the presence of 9-cis retinoic acid, attenuate HNF4a/ARP-1-mediated activation. We analyzed direct interactions of HNF4a, ARP-1, RARa and RXRa with wild type and mutant DR1 and DR4 elements using electrophoretic mobility shift assays (EMSA) with nuclear extracts from human hepatoma HuH7 cells and recombinant HNF4a and ARP-1 overexpressed in COS7 cells. EMSA and supershift of bands with specific antibodies showed that homodimers of HNF4a bind the 5 and 3 half-sites of DR1, while ARP-1 monomers bind exclusively to its 5 half-site, with dissociation constants (Kd) of 14 nM and 1.5 nM, respectively. These results suggested that HNF4a/ARP-1 heterodimers bind to DR1 and activate the *LIPC* promoter, with ARP-1 occupying the 5 and HNF4a the 3 half-site. RARa/RXRa heterodimers also bind to the DR1 element with equal affinity as HNF4a and ARP-1. HNF4a and ARP-1 bind independently to the 5 half-site of DR4 with Kd of 14 and 3 nM, respectively, suggesting that excess ARP-1 binds to the 5 half-site of DR4 and suppresses HNF4a/ARP-1-DR1-mediated co-activation. We confirmed an essential role for HNF4a in *LIPC* expression in vivo by showing that liver-specific targeted disruption of HNF4a gene in mice prevents liver expression of HL mRNA. We conclude that HNF4a is necessary but not sufficient for activation of *LIPC* expression in hepatocytes, and that the intracellular balance between HNF4a, ARP-1, RARa and RXRa controls its expression by interacting with the DR1 and DR4 promoter motifs.

**In vivo analysis of protein-DNA interactions in the  $\gamma$ -globin gene locus.** *K.F. Vieira, J. Bungert.* Biochemistry and Molecular Biology University of Florida, Gainesville, FL.

The human  $\gamma$ -globin genes are expressed at high-levels in erythroid cells. This high-level expression is dependent on the presence of the locus control region (LCR), which is 8-20 kb upstream of the  $\gamma$ -globin genes. Recent data support the hypothesis that transcription complexes are recruited to the LCR and to the genes. It has been suggested that the LCR is the primary attachment site for transcription complexes, which are subsequently transferred to individual globin genes. To investigate the order of recruitment of RNA polymerase II and other factors to the active gene promoters and to the LCR, erythroid cells were synchronized at the border of G1 and S phase of the cell cycle using the double thymidine block method. These cells were then released from the block and used for ChIP analysis. The data show that before release from the block, members of the transcription machinery were present both at the active gene promoter and within the LCR. Within early S phase no factors were present at the active gene promoter, while they could still be detected at the LCR. Later in S phase there is faint detection of the factors at the active gene promoter, and less at the LCR. By late S phase, the factors have returned to both locations. Also, it seems that NF-E2 returns first, followed by the transcription machinery. DNA analysis using multiplex PCR suggests that replication of the active globin gene is likely to have occurred between 45 minutes and 2 hours into S phase. Taken together, our data supports a model by which the transcription machinery is first recruited to the LCR and subsequently transferred to the active gene promoters.

***In vivo* protein-DNA interactions analysis using dimethylsulfate, UVC and DNaseI at the murine survival motor neuron gene promoter.** F. Vigneault<sup>1</sup>, R. Rouget<sup>2</sup>, C. Rochette<sup>2</sup>, S. Morissette<sup>2</sup>, C. Codio<sup>2</sup>, I. Paradis<sup>1</sup>, L.R. Simard<sup>2</sup>, R. Drouin<sup>1</sup>. 1) Research Center, Hospital Saint-Francois d'Assise, CHUQ, Laval University, Quebec, QC, Canada, G1L 3L5; 2) Research Center, Hospital Sainte-Justine, University of Montreal, Montreal, QC, Canada.

In humans, loss or mutation of the Survival Motor Neuron (SMN) gene is responsible for proximal spinal muscular atrophy, the second most common autosomal recessive disease of childhood after cystic fibrosis. Previous studies suggest that in mouse, the *Smn* gene is subject to temporal and spatial regulation both at the level of transcription and post-translational modification. In order to define the regulatory elements that account for the control of *Smn* gene expression, we have conducted *in vivo* footprinting analysis of the *Smn* gene promoter region in the P19 embryonal carcinoma cells, as they can be induced to differentiate into neuronal-like cells by retinoic acid. To do so, we have used DMS, UVC and DNaseI as probing agents to characterize the *Smn* promoter using ligation-mediated polymerase chain reaction. Systematic comparison of the footprints obtained from differentiated and undifferentiated P19 EC cells does not explain the down-regulation of the promoter activity for the sequence studied. Footprints were observed at putative sites of sequence-specific DNA binding proteins like Sp1, AP-2, H4TF2, ADR1, GABP, Elk-1, NFIL-6 RE, GAGA PEA-3, Ets-1, GABP, PU.1, MEF-2, E1A-F. *In vitro* studies by transient transfection experiments with a reporter gene and EMSA experiments have confirmed that Sp1-like (AP-2), NFIL6-RE and Ets (PEA-3/Ets-1) cis-elements are essential for the core *Smn* promoter activity in undifferentiated P19 cells; whereas only the NFIL6-RE and Ets sites were required in differentiated P19 cells. *In vivo* footprinting analysis results confirmed most of those interactions but abrogate some like the AP-2 interaction. Furthermore, some other footprints were detected on sequences that do not correspond to any known transcription factor binding sites. These results demonstrate the importance of *in vivo* studies as *in vitro* studies do not exactly reflect DNA-protein interactions implicated in gene regulation.

**A frameshifting mutation causes skipping of a preceding exon and modifies the effects of nonsense-mediated mRNA decay (NMD).** *K. Ohno, M. Milone, J.M. Brengman, X.-M. Shen, A.G. Engel.* Dept Neurology, Mayo Clinic, Rochester, MN.

Nonsense-mediated altered splicing affects splice site selection of a mutation-bearing exon, but not of a remote exon. We here report that a frameshifting 7-bp deletion (553del7) in exon 7 of *CHRNE* encoding the acetylcholine receptor subunit, observed in seven congenital myasthenic syndrome patients, unexpectedly causes skipping of the preceding 101-bp exon 6 in muscle. Skipping of exon 6 restores the open reading frame after 553del7. To understand how the preceding exon is skipped in *CHRNE*, we cloned the entire *CHRNE* spanning 12 exons and 11 introns, engineered a series of *CHRNE* mutants, transfected wild-type and mutant constructs into COS cells, and analyzed pre-mRNA splicing by RT-PCR and by the ribonuclease protection assay.

Transfected COS cells showed the same aberrant splicing that was detected in the patient muscles. When NMD was inhibited by anisomycin, even wild-type *CHRNE* produced an exon 6-skipped transcript, but optimization of splicing signals for exon 6 prevented skipping of exon 6. This indicates that inherently weak splicing signals at the boundaries of exon 6 results in skipping of exon 6 even in wild-type *CHRNE*, but the transcript is degraded by NMD. Therefore, the consequences of the weak splicing signals for exon 6 are corrected by NMD, which removes the abnormal transcript arising from wild-type *CHRNE*. In contrast, an exon 6-skipped transcript harboring 553del7 has no premature stop codon, and is immune to NMD, whereas the normally spliced transcript harboring 553del7 is degraded by NMD. Therefore, in the presence of 553del7, NMD works only on the normally spliced transcript, and the exon 6-skipped transcript remains intact. A similar mechanism likely causes skipping of remote exons in other genes.

Presence of weak splicing signals for *CHRNE* exon 6 also prompted us to search for mutations in exon 6 that disrupt an exonic splicing enhancer. Indeed, we found that EF157V and E154X in exon 6, observed in two other patients, cause aberrant splicing of exon 6.

**Activation of exon incorporation into mRNA by SAP 145 or Tat-SF1 in *in vitro* mini-dystrophin splicing.** A. Surono, Y. Takeshima, M. Matsuo. Dept Pediatrics, Kobe Univ Grad Sch Medicine, Kobe, Hyogo, Japan.

Exonic splicing enhancers (ESEs) are RNA sequences required for accurate splicing that removes intron sequence from pre-mRNA. ESEs are believed to exert its function through its association with nuclear protein. In the previous study, we characterized the 5' part of deleted sequence of the dystrophin Kobe as ESE and named as ESE-19. We have isolated the nuclear proteins bound to ESE-19 by using two-step chromatography. Amino acid analysis revealed two known proteins of spliceosome-associated protein 145 (SAP145) and Tat-SF1, the essential splicing factor assembled on the branch point site and general transcription elongation factor, respectively. The binding characters were examined using recombinant SAP145 (rSAP145) and Tat-SF1 (rTat-SF1). The interacting rSAP145 with ESE-19 was enhanced by rTat-SF1 in a dose dependent manner. In addition, the binding ability of ESE-19 to nuclear proteins was strongly enhanced by addition of individual rSAP145 and rTat-SF1. The rSAP145 and ESE-19 interaction was abrogated completely by using mutant ESE-19 and antisense RNA complementary to ESE-19. We also revealed that 8 nucleotides at the 5' end of ESE-19 is a novel binding site of SAP145. In order to see the function of rSAP145 and rTat-SF1, *in vitro* mini-dystrophin splicing system was used. Surprisingly, either rSAP145 or rTat-SF1 could activate incorporation of exon of mutant dystrophin minigenes into mRNA in a dose dependent manner, indicating a direct involvement of both proteins on splicing activation. Our results show a nuclear proteins binds to ESE-19 and these proteins enhance exon incorporation.

These provide important new insight into how SAP145 interacts with ESE in addition to be present in spliceosome assembly on the branch point site and its association with Tat-SF1 on splicing regulation of dystrophin mRNA.

**Novel alternatively spliced forms found for seven genes in transcriptional profiling of asthma candidate genes in**

**11 asthma related cell lines and lung tissue.** A. Polvi<sup>1</sup>, S. Ruosaari<sup>2</sup>, J. Vendelin<sup>1,3</sup>, A. West<sup>4</sup>, I. Saarikko<sup>4</sup>, A. Reinikainen<sup>4</sup>, J. Hollmn<sup>2</sup>, T. Laitinen<sup>1,3</sup>, H. Mannila<sup>2</sup>, R. Lahesmaa<sup>4</sup>, J. Kere<sup>1,3,5</sup>. 1) Department of Medical Genetics, Biomedicum Helsinki, University of Helsinki, Finland; 2) Laboratory of Computer and Information Science, Helsinki University of Technology, Finland; 3) Geneos oy, Helsinki, Finland; 4) Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, Turku, Finland; 5) Karolinska Institute, Department of Biosciences at Novum, Clinical Research Center, Sweden.

To study expression of a large group of asthma susceptibility genes simultaneously in cell types relevant to asthma pathogenesis we used a microarray with 2225 asthma/immunology related genes and a representative panel of 11 cell lines and lung tissue. Cells included Jurkat, Ramos, U-937, HMC-1, clone 15 HL-60 (15 HL-60), A549/NCI-H358, IMR-90/CCD-25Lu, BE(2)-C and RD to represent T-lymphocytes, B-lymphocytes, monocytes, mast cells, eosinophils, epithelial cells, fibroblasts, nerve cells and muscle cells, respectively. We determined cell line- and cell type-specific genes using bootstrap analysis, database analysis and RT-PCR. Previously undetected expression of several genes was found in many cell lines. Also, novel alternatively spliced forms were found for seven of 34 genes studied by RT-PCR (21%), for CDS1, MX2, CDC7L1, TNFSF10/TRAIL, AQP1, FOXF1/FREAC1 and CCL4/SCYA4/ACT-2. Alternatively spliced forms were generally expressed in same cell lines, but weaker than full length forms. Only AQP1 splice variants had different tissue distribution. According to sequence, alternative splicing excluded exon 11 from CDC7L transcript, but did not change the reading frame. In transcripts of five genes, reading frame and the coded amino acid sequence of the rest of the protein also changed in the alternative spliced site. Surprisingly high percentage of novel alternatively spliced forms for genes were found in a usual RT-PCR amplification experiment. In general, alternatively spliced forms and their importance should be more carefully studied.

**Purification and Characterization of Vaccinia Expressed FLAG-ATM Protein.** *H.H. Chun<sup>1</sup>, R.A. Cary<sup>2</sup>, D.J. Rawlings<sup>3</sup>, R.A. Gatti<sup>1</sup>.* 1) Department of Pathology, The David Geffen School of Medicine at UCLA, Los Angeles, CA 90095; 2) Biosciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545; 3) Department of Pediatrics, University of Washington School of Medicine, Seattle, WA 98195.

The ATM gene product plays an important role in responding to double strand DNA breaks produced during cellular metabolism or by environmental agents. Some biochemical studies of ATM function have been hampered by the lack of efficient expression systems and purified ATM protein. We report the construction of a vaccinia virus expressing ATM, vWR-ATM, which is used to produce large amounts of functional FLAG-tagged ATM protein (FLAG-ATM) in HeLa cells. *In vivo* assays show expression of FLAG-ATM protein in an ATM-deficient cell line infected by vWR-ATM, and functional activity, as measured by p53 Ser15 phosphorylation. Purification of FLAG-ATM, from  $8 \times 10^6$  HeLa cells infected with vWR-ATM, was achieved through binding to a FLAG affinity resin and elution by peptide competition. We recovered about 40 mg of FLAG-ATM, where the majority of purified protein was ATM. Kinase assays using purified FLAG-ATM showed that GST-p53 serine 15 phosphorylation increased in the presence of DNA double strand breaks when compared to reactions without DNA or those with DNA containing no breaks. FLAG-ATM kinase activity was dependent on manganese and independent of magnesium. Atomic force microscopy (AFM) of FLAG-ATM with linear DNA showed FLAG-ATM binding to DNA with a preference for DNA ends. Immunoblotting after purification showed that FLAG-ATM was phosphorylated at Ser1981, which was removed after phosphatase treatment. Purification of FLAG-ATM from vWR-ATM infected cells has produced the highest yield of purified protein reported to date.

**Splice site mutations in the *Jagged1* Gene.** A.O. Laney, J.J.D. Morrissette, J. Abed, J. Wound, N.B. Spinner. Children's Hospital of Philadelphia & University of Pennsylvania School of Medicine, Philadelphia, PA.

Mutations in *Jagged 1* cause the autosomal dominant disorder Alagille syndrome, and some forms of isolated cardiac disease. The majority of mutations are protein truncating, with a smaller percentage being missense (10%), splicing (10%) or total gene deletions (5-7%). Mutations in the invariant nucleotides of the splicing consensus sequence always lead to abnormal RNA production, however, there mutations near these sequences, and even some mutations in exons have been shown to lead to abnormal splicing. We have studied potential splicing mutations (in invariant and consensus sequence nucleotides) to determine their impact. Determination of the impact of mutations on splicing requires analysis of cDNA, as outcome cannot always be predicted.

Eleven patients were studied by preparing cDNA from RNA after treatment with cycloheximide to stabilize the RNA. Selected regions of the *Jagged1* gene were then sequenced. Five patients had mutations in the invariant splicing consensus sequences (GT or AG at intron exon boundaries) and 7 patients had mutations further from the invariant positions, but within the general splicing consensus region. All 5 patients with mutations in the invariant nucleotides demonstrated abnormal splicing. These included abnormal splicing of exons 3, 6, 11 and 21.

Of the seven patients with potential mutations in the consensus sequence, 1349-12T>G was found to cause abnormal splicing of exon 11, and 2458+5G>A caused abnormal splicing of exon 20. Three other variants did not result in abnormal products, 1721-21G>A, 1121-25A>G and 1349-11T>G. It is noteworthy that two mutations (1349-12T>G and 1349-11T>G) were only a single base apart, both resulted in loss of a T from the splice acceptor region, yet only 1349-11T>G caused abnormalities, while the change in the minus 12 position resulted in a normal product.

**Abnormal subcellular localization and apoptotic cell death of mutant ELOVL4, the gene for Stargardt-like macular dystrophy (STGD3).** *G. Karan, N. Faulkner, M. Payne, M. Machan, S. Thirumalaichary, S. Sims, L. Jiang, Z. Yang, K. Zhang.* Department of Ophthalmology and Visual Science, Programme in Human Molecular Biology and Genetics, University of Utah Health Science Center, Salt Lake City, UT 84112.

**Purpose :** *ELOVL4* belongs to fatty acid elongase (*ELO*) family of genes. Members of the *ELOVL4* family share common structural features, including multiple putative membrane-spanning domains, a single histidine cluster motif and putative dilysine motifs thought to signal ER retention. A 5 bp deletion of human *ELOVL4* causes autosomal dominant stargardt like macular dystrophy (STGD3). This deletion results in a frame-shift and loss of C-terminal 51 amino acids that include the dilysine ER targeting signal. The goals of this study are to determine the subcellular localization of wild type and mutant *ELOVL4* proteins in the transfected cells. **Methods :** Both wild type and mutant *ELOVL4* cDNAs were cloned in a pEGFPC1 expression vector. The resultant recombinant constructs express EGFP-*ELOVL4* fusion proteins. Each plasmid construct was used to transfect NIH 3T3, HEK293, COS7 and primary retina cells. To determine the subcellular localization of EGFP-*ELOVL4* fusion proteins, a series of markers for ER, mitochondria and peroxisomes were used. The transfected cells were viewed using confocal and episcopic-fluorescence microscopy. Western blotting was performed to analyze the expression of fusion proteins using anti-GFP antibody. **Results :** We expressed EGFP-*ELOVL4* proteins in NIH 3T3, HEK293, COS7 and primary retina cells. Wild type EGFP-*ELOVL4* protein was localized in the ER compartment. The mutant EGFP-*ELOVL4* did not distribute uniformly in ER, but appeared to localize into spots or pools throughout transfected cells. Cells expressing mutant EGFP-*ELOVL4* underwent apoptotic cell death. **Conclusions :** Wild type EGFP-*ELOVL4* is localized to ER, as predicted from its primary sequence. Mutant EGFP-*ELOVL4* protein has a different localization and causes apoptotic death. The above results could be an explanation of the photoreceptor cell degeneration in patients with STGD3.

**Cloning and characterization of a human gene (*rwd*) encoding a RING finger and WD-40 repeats containing protein.** W. Huang, J.K. Kane, M.D. Li. Dept. of Psychiatry, Univ. of Texas, HSC, San Antonio, TX 78229.

Recently we identified a novel human gene (*rwd*), which encodes a RING finger and WD-40 repeats containing protein, from a human brain cDNA library. Sequence analysis indicated that the complete mRNA is approximately 4.5 kb. Northern blotting analysis showed that the gene is expressed in several human tissues, most abundantly in brain and testis. Additionally, a transcript of approximately 7.5 kb was detected in human brain only. Radiation hybrid mapping analysis demonstrated the gene is located on chromosome 3q26, which was further confirmed by BLAST searching of human genome sequence. The genomic structure of *rwd* gene was characterized to span over 200 kb region with 19 exons. The transcription initial site of *rwd* gene was determined by primer extension. And its promoter features a typical housekeeping gene, with several putative GC box motifs for the binding of SP1 transcription factor family, but lacking a classical TATA box motif. The predicted protein RWD consists of 1,008 amino acid residues with RING finger, B-box, coiled-coil domains in N terminus and WD-40 repeats domain in C terminus. Western blotting analysis indicated an expected protein of approximately 110 kD was detected in the human brain extract and several human cell lines. Cellular localization studies with expressed protein tagged in the N terminus with HA or enhanced green fluorescent protein demonstrated the protein is localized in the cytoplasm of human HEK293 cell line. Functional studies are under way to further elucidate its biological role and its potential involvement in the etiology of human disease(s). (supported by DA-12844 and DA-13783).

**TBX5, a gene mutated in Holt-Oram Syndrome, is regulated through a GC box and T-box binding elements.** *G. Sun<sup>1</sup>, L. Scott<sup>1</sup>, X. Huang<sup>1</sup>, Q. Nguyen<sup>1</sup>, C. Price<sup>1</sup>, T. Huang<sup>1,2</sup>*. 1) Department of Pediatrics, University of California, Irvine, Irvine, CA; 2) Developmental and Cell Biology, University of California, Irvine, CA.

TBX5 is a member of the T-box gene family and encodes a transcription factor that regulates the expression of other gene(s) in the developing heart and limbs. Mutations of TBX5 cause Holt-Oram syndrome (HOS), an autosomal dominant condition characterized by congenital heart defects and limb anomalies. How TBX5 gene expression is regulated is still largely unknown. In order to identify transcription factors regulating TBX5 expression, we examined the 5'-flanking region of the human TBX5 gene. We determined that up to 300 of the 5'-flanking region of the TBX5 gene was necessary for promoter activity in ECL2 cells. One GC box, three potential T-box binding sites (TBX5-A, TBX5-B, and TBX5-C), and one NKX2.5 binding site were identified. Site-directed mutagenesis of the potential binding sites revealed that the GC box, TBX5-B, TBX5-C, and NKX2.5 are functionally positive for the expression of TBX5. DNA footprint analysis showed that these binding regions are resistant to DNase I digestion. Electrophoretic Mobility Shift Assays further demonstrated the protein-DNA interactions at the GC box and the potential TBX5-B, TBX5-C and NKX2.5 sites in a sequence-specific manner. The ability of TBX5 to regulate its own promoter was further demonstrated by the ability of ectopically expressed human TBX5 to increase reporter expression. We conclude that the GC box, T-box binding sites and NKX2.5 binding sites play important roles in the regulation of TBX5 expression, and that TBX5 is likely to be autoregulated as part of the mechanism of its transcription.

**Mutation Studies and Ocular Localization of Optineurin Protein in Primary Open Angle Glaucoma (POAG).** *T. Rezaie*<sup>1</sup>, *A. Child*<sup>2</sup>, *R. Ritch*<sup>3</sup>, *T. Krupin*<sup>4</sup>, *J. Liebmann*<sup>3</sup>, *E. Ilitchev*<sup>3</sup>, *M. Sarfarazi*<sup>1</sup>. 1) Molecular Ophthalmic Genetics, Univ Connecticut Health Ctr, Farmington, CT; 2) St. Georges Hospital, London, UK; 3) New York Eye and Ear Infirmary, NY; 4) University Eye Specialists, Chicago, IL.

We have previously reported mutations in Optineurin (*OPTN*) in a group of families with adult-onset POAG (*Science* 2002; 295:1077). Subsequently, we sequenced a total of 76 familial (including 22 high-pressure glaucoma, HPG) and 206 sporadic LPG (low-pressure glaucoma) patients for the entire of *OPTN* gene. Altogether, we identified 6 mutations, 1 susceptibility allele and several other SNP variants. Extensive genotyping of 7 families with E50K mutation over a region of 2.65 Mb confirmed that E50K is a recurrent mutation. Overall, we identified mutations in 13% of familial and 3% of LPG sporadic cases. One risk-associated factor (M98K) was observed in 12% of familial, 16% of sporadic LPG and 2% of normal controls. Therefore, mutations in the *OPTN* gene are more frequently observed in familial and LPG patients than, sporadic and HPG cases, respectively. This is also in a general agreement with previously reported mutations in *CYP1B1* and *MYOC* in patients with Primary Congenital Glaucoma and JOAG/POAG, respectively. By Western blotting, we detected *OPTN* protein in aqueous humor samples from human and 7 other species. By immunohistochemistry, we further studied ocular localization of *OPTN* protein in human, Rhesus monkey and mouse eyes. In anterior segments, positive staining was observed with non-pigmented ciliary epithelium, ciliary muscle, lens epithelium, iris constrictor muscle, endothelial cells of blood vessels and Schlemm's canals. In retina, intense positive labeling detected in nerve fiber layers, ganglion cells, inner and outer plexiform layers and pigmented epithelium. In monkey and mouse, axons of optic nerve ganglion cells, glial cells and endothelial cells of optic nerve blood vessels were also stained. Immunocytochemistry showed positive labeling in human optic nerve head astrocytes obtained from normal and glaucomatous eyes. In conclusion, Optineurin protein expressed in various ocular tissues and *OPTN* mutations mainly observed in patients with LPG. Supported By: EY-09947.

**TRPM5 is a transient calcium-activated cation channel.** *D. Prawitt<sup>1</sup>, L. Brixel<sup>1</sup>, M.K. Monteilh-Zoller<sup>2</sup>, C. Spangenberg<sup>1</sup>, A. Fleig<sup>2</sup>, B.U. Zabel<sup>1</sup>, R. Penner<sup>2</sup>.* 1) Molecular Genetics Laboratory, Children's Hospital/ University of Mainz, 55131 Mainz, Germany; 2) Laboratory of Cell and Molecular Signaling, Center for Biomedical Research at The Queens Medical Center and John A. Burns School of Medicine University of Hawaii, Honolulu, HI 96813, U.S.A.

Transient receptor potential (TRP) proteins are a diverse family of proteins with structural features typical of ion channels. They are grouped into three subfamilies, named after their founding members TRPC (canonical TRPs), TRPV (Vanilloid receptor like) and TRPM (Melastatin like). To date the human TRPM subfamily consists of eight members with partly unknown physiological function but possible involvement in human disease. We cloned and initially described *TRPM5*, a member of the TRPM subfamily, which plays an important role in taste receptors (Perez et al., 2002 and Zhang et al., 2003), although its activation mechanism remains controversial and its function in signal transduction is unknown. We demonstrate that *TRPM5* is expressed as a 4.5 kb transcript not solely in the gustatory cells, but in a variety of fetal and adult tissues. To further elucidate the biological function of *TRPM5* we studied the exact activation mechanism of this protein by establishing stable transgenic HEK293 cell lines with the human *TRPM5* and *TRPM5*-EGFP fusion constructs. The latter enabled us to localize the protein to the cell membrane, suggesting a role as a functional channel. We then characterized the electrophysiological properties of the *TRPM5* channel by using whole cell recordings and excised membrane patch-clamp analysis. *TRPM5* displays characteristics of a CAN (Calcium-activated-nonselective) cation channel. It is directly activated by  $[Ca^{2+}]_i$ , but seems not to conduct  $Ca^{2+}$  ions. Instead it carries monovalent ions ( $Na^+$ ,  $K^+$ ) after receptor mediated cytosolic  $Ca^{2+}$  increase, thereby modulating the membrane potential (depolarisation) with effects in cells with non-excitabile and excitabile membranes. We could also show the specific electrophysiological properties of the channel in cells with endogenous *TRPM5*, supporting the proposed functional involvement in the conversion of a chemical stimulus into an electrical signal.

**Characterization of a novel brain specific transcript on human chromosome 20q13.** *S. Worch, I. Hansmann, D. Schlote.* Institute of Human Genetics, Halle, Germany.

Based on human EST-markers we have identified a novel human gene on HSA20q13.32 whose expression appears to be restricted to the brain as Northern analysis of 8 human tissues revealed expression of a 3.2 kb and a 3.0 kb transcript in brain only. One corresponding cDNA (AJ311122) contains a 1680 bp ORF distributed on 13 exons, a second one as part of the EMBL sequence database has a different initial exon resulting in a 1671 bp ORF. Both ORFs show a homology of the endmost 1557 bp beginning in exon 2. Multiple tissue Northern analysis of the corresponding mouse transcripts has revealed 3 major signals of 3.3 kb, 2.9 kb and 2.5 kb visible in brain only emphasizing the tissue specificity of the human expression pattern. Analysis of the 5'-end of the transcribed RNA by RACE-PCR yielded four initial exons suggesting, as in man, alternative splicing. These four different mRNAs of the gene, mapping to mouse chromosome 2H4, could be verified by RT-PCR. During detailed expression analysis using 8 different mouse brain specific tissues a complex tissue specific pattern has been detected concerning the quantity of individual transcripts. A developmental specific expression pattern has been found during embryogenesis displaying weak signals from day 10 pc and strong signals from day 15 pc onwards to adult mice suggesting a function from late development. Further on, this complex expression pattern is not restricted to the 5'-end as we could demonstrate alternative polyadenylation in hybridization experiments. The transcripts vary in ORF size but all show the 1557 bp homology as found in man. Searching the databases a striking homology of approximately 120 aa at the C-terminal end of the predicted protein was found shared with 3 other human proteins with so far unknown function, suggesting a putative domain which seems to be highly conserved down to *D. melanogaster* and *C. elegans*. Therefore further experiments should lead to characterization of the gene product, possibly relevant in brain development or function.

**Fanconi Anemia and oxidative stress: The FANCG protein interacts with Peroxiredoxin 3.** *S.E. Plon<sup>1,2</sup>, K. Leung<sup>1</sup>, S.S. Mukhopadhyay<sup>1</sup>, H. Youssoufian<sup>2,3</sup>*. 1) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 2) Dept Molecular and Human Genetics, Baylor Col Medicine, Houston, TX; 3) Bristol-Myers Squibb CO, Princeton, NJ.

Fanconi anemia (FA) is an autosomal recessive disorder that results in bone marrow failure and increased susceptibility to cancer. Currently, mutations in eight distinct genes, labeled complementation groups A-G can result in FA. FANCA, C, F, and G proteins have been shown to interact together by two-hybrid and biochemical methods, forming a nuclear protein complex needed to activate FANCD2 in response to DNA damage. Several reports have also linked FANCC and FANCG to proteins in the cytochrome P-450 pathway, indicating their potential roles in oxidative stress. In order to further identify binding partners of the FA proteins we performed a yeast two-hybrid screen utilizing a bait construct containing the carboxy terminus of the FANCG protein (amino acids 300-622) and a human B-lymphocyte cDNA prey library. One of the positive interacting clones contained a cDNA encoding peroxiredoxin 3 (PRDX3), a member of the peroxiredoxin family of proteins possessing peroxide reductase activity which protects against oxidant injury. PRDX3 is regulated by c-myc and has been observed to be overexpressed in breast cancer and hepatocarcinoma cells. The positive interaction in yeast was shown to be dependent on the presence of both the PRDX3 and the FANCG encoding plasmids. Two different rabbit polyclonal antibodies to PRDX3 were raised. Co-immunoprecipitation assays utilizing protein extracts from undamaged COS-1 cells reveal that the endogenous PRDX3 protein can interact with an exogenously expressed HA-epitope tagged FANCG protein. These results provide additional evidence that Fanconi Anemia proteins interact with cellular proteins that regulate the response to oxidative damage.

**Microarray analysis of mouse forebrain gene expression after TCDD exposure.** *H. Fujita*<sup>1</sup>, *T. Mitsuhashi*<sup>1</sup>, *J. Yonemoto*<sup>2</sup>, *T. Takahashi*<sup>1</sup>, *K. Kosaki*<sup>1</sup>. 1) Dept Pediatrics, Keio Univ Sch Med, Tokyo, Japan; 2) Environmental Health Sciences Division, National Institute for Environmental Studies, Tsukuba, Japan.

Dioxins have been shown to possess developmental toxicity on mammals. The effects of dioxins are mediated via the aryl hydrocarbon receptor. The dioxin-aryl hydrocarbon receptor complex binds to the cognate xenobiotic responsive elements in the promoter regions of the target genes. Most downstream target genes of the dioxin receptor are unknown. Recently, we demonstrated that *in utero* exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) delays neocortical neurogenesis in mice. Using the Affymetrix cDNA microarray, we evaluated overall change of expression profile in the fetal forebrain following dioxin exposure to identify potential downstream target genes of the aryl hydrocarbon receptor. A single oral dose of TCDD (20 mcg /kg body weight) was administered to pregnant C57BL/6 mice on gestation day (GD) 7. Three pups were sacrificed and their forebrains were collected on GD12. DNA was prepared from the forebrains of dioxin-exposed mice and non-exposed mice, and was hybridized on the Murine genome U74Av2 array (Affymetrix) which carries more than 10,000 genes. In comparison with the control, seven genes were expressed more than two fold in the forebrain of the exposed pups. Among the 7 genes were *Cyp1a1* and *Cyp1b1*, which are already known to be induced by dioxins, *BSCv*, and LIM homeobox protein *Lhx8*. *BSCv* is a gene homologous to plant strictosidine synthase, a member of the metabolic pathway of indole alkaloids. Prenatal exposure to dioxins leads to cleft palate. It is interesting to note that loss-of-function mutations in *Lhx8* also lead to the same malformation. Teratogenic action of dioxin on palate formation might be mediated by abnormal expression of *Lhx8*.

Program Nr: 1080 from 2003 ASHG Annual Meeting

**A computational survey of young Alu subfamilies within non-human primate genomes.** *M. Carroll.* Department of Chemistry, Xavier University, New Orleans, Louisiana.

We have employed computational genomics to survey non-human primate genomic DNA sequence databases to determine a unique subfamily sequence diversity among recently integrated Alu Y elements and to measure Alu polymorphism among unrelated non-human primates. Alu elements are the most abundant Short INterspersed Elements (SINEs) at over one million copies in the human genome. Alu repeats compose greater than 10% of the mass of the human genome. *Pan troglodyte*, the common chimpanzee, is believed one of the closest living ancestors to humans. Its genome is 95-99% identical to the human genome and is a likely place to find clues to the dynamics of Alu insertion and evidence of the influence of Alu retroposition in genome structure and gene distribution. The youngest subfamilies of Alu elements, Ya8, Ya5, Yb8, appeared in the human genome between 2.5 to 7 million years ago after the divergence of the human and non-human primate genomes. These young subfamilies are absent from non-human primates. We assert that a similar appearance and pattern of mobile element subfamily structure and distribution exists in non-human primates. The purpose of this study is two-fold: to identify and describe using the available non-human primate genome a young Alu subfamily structure by describing diagnostic nucleotide patterns among Alu Y elements; and to amplify these sequences for a preliminary assessment of Alu polymorphisms among a small population of non-human primates. This is accomplished using the InfoMax VectorNTI Advance software suite and PCR. We are encouraged by the efforts to sequence non-human primate genomes because enormous benefit to understanding human genome structure and gene distribution will result.

**AUG-independent translation of human LINE-1 ORF2.** *R.S. Alisch, J.L. Garcia-Perez, J.V. Moran.* Dept Human Gen & Internal Med, Univ Michigan, Ann Arbor, MI.

Retrotransposition-competent L1s contain two open reading frames (ORF1 and ORF2), which are separated by a 66 nucleotide inter-ORF sequence that includes two in-frame stop codons. Previous studies demonstrated that both proteins are required for efficient retrotransposition (RTSN) in cultured human cells. Here, we have conducted a genetic study to determine how ORF2 is translated. Deletion analysis shows that neither the inter-ORF sequence nor the carboxyl-terminus of ORF1p is required for RTSN. Similarly, we showed that the putative ORF2 AUG could be mutated to any other amino acid without drastically affecting RTSN (i.e., the resultant constructs retrotransposed at 10-70% wild type levels). In contrast, changing the AUG to a stop codon (M1X) reduced RTSN to <2% wild type levels. Since there are no other initiation codons near the amino terminus of L1 ORF2p, our data suggest that ORF2 can be translated in an AUG-independent manner. To further explore how ORF2 is translated, we placed in-frame stop codons (i.e., TG23XX) or a frame-shift induced stop codon (i.e., T2X) at the same putative amino acid position of ORF2p. Paradoxically, the in-frame stop codons nearly eliminated L1 RTSN, whereas the frame-shift induced stop codon only reduced RTSN by ~2-fold. Interestingly, while the frame-shift induced stop codon interrupts the ORF2 reading frame in the 5'-3' direction, it still allows for a continuous ORF if we scan backwards (i.e., in the 3'-5' direction) from a conserved Asn residue (N14) required for L1 EN function. Thus, we proposed that L1 mRNA contains a downstream landing pad that positions incoming ribosomes at position one of ORF2. To test this hypothesis, we inserted a single guanosine nucleotide immediately downstream of each of the M1X stop codon mutations described above. Consistent with our hypothesis, each of the resultant constructs retrotransposed at efficiencies ~10-fold higher than the original M1X mutants. Thus, our data suggest that L1 ORF2 translation either occurs by an unusual mechanism such as ribosome shunting or is facilitated by an unconventional internal ribosome entry sequence. Indeed, our data demonstrate a novel form of gene regulation in human cells.

**Direct visualization of L1 ORF2 protein in human cells.** *J.L. Goodier<sup>1</sup>, K.A. Engleka<sup>1,2</sup>, E.M. Ostertag<sup>1</sup>, M.C. Seleme<sup>1</sup>, H.H. Kazazian<sup>1</sup>.* 1) Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PAC; 2) Division of Cardiology, University of Pennsylvania School of Medicine, Philadelphia, PAO.

The human L1 retrotransposon contains two open reading frames separated by a short intergenic spacer. ORF1 encodes a 40 kDa protein which binds L1 RNA to form cytoplasmic RNPs. The 150 kDa ORF2 protein has endonuclease and reverse transcriptase activities and is expressed at very low levels in comparison to ORF1. Functional analyses have been hampered by difficulties detecting ORF2 protein. We report detection of both ORF1 and ORF2 proteins in cultured human cells following their overexpression using a vaccinia virus/T7 RNA polymerase hybrid expression system. Epitope-tagged forms of overexpressed ORF1 and ORF2 were detected by immunoblotting using antibodies against the epitope, and co-migrated with the respective ORF proteins produced in a baculovirus system. Furthermore, antibodies raised against an ORF2 amino-terminus peptide revealed several bands, including full length protein. However, antibodies against an ORF2 carboxy-terminal peptide detected only full length protein. This is consistent with the generation of truncated ORF2p forms lacking a carboxy-terminus. ORF2 overexpression appeared toxic to cells, and ORF2p containing mutations in the endonuclease and/or reverse transcriptase domains was detected in significantly greater amounts than wild-type protein. We used the vaccinia virus/T7 RNA polymerase hybrid system to track subcellular localization of L1 proteins, both as fusions with green fluorescent protein (GFP) and by immunocytochemistry. ORF1p localizes in the cytoplasm with a speckled pattern. ORF2p is also cytoplasmic but without speckled patterning, even when coexpressed with ORF1p from a bicistronic construct. Furthermore, ORF2p is detected in nucleoli in a significant percentage of cells. Data suggest that C-terminally-deleted but not full length ORF2 protein is directed to nucleoli. Putative nucleolar localization signals were identified. This work represents the first direct visualization of ORF2p within mammalian cells.

**Nonsense surveillance regulates diverse classes of physiologic transcripts and mutes a noisy genome. *J.T.***

*Mendell*<sup>1,2</sup>, *N.A. Sharifi*<sup>1</sup>, *H.C. Dietz*<sup>1,2</sup>. 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) HHMI.

Transcripts containing premature termination codons (PTCs) are rapidly degraded in eukaryotic cells through nonsense-mediated mRNA decay (NMD). In mammals, PTCs can also induce nonsense-mediated altered splicing (NAS), a complementary pathway through which nonsense codons influence pre-mRNA processing. Although these pathways have the ability to modulate the phenotype arising from nonsense or frameshift mutations, remarkably little is known regarding the physiologic role of NMD and NAS. To address this issue, we used RNA interference in mammalian cells to silence expression of *rent1/hUpf1*, a factor essential for both NMD and NAS, and performed expression profiling using Affymetrix microarrays. Of the approximately 5000 assayed transcripts, 4% were upregulated at least 2-fold and 4% were down-regulated at least 2-fold. Transcripts that were upregulated in response to NMD inhibition could be stratified into broad classes: transcripts with introns in the 3' untranslated region, alternative PTC-encoding exons, or upstream open reading frames; transcripts that encode selenocysteine-containing proteins; and transcripts derived from pseudogenes, ancient transposon families, or endogenous retroviruses. These physiologic substrates are unified by the presence of a spliced intron downstream of a termination codon, a context known to be sufficient to initiate NMD. In order to confirm that these transcripts are primary substrates for the NMD pathway, mRNA decay rates were determined for selected examples. The vast majority of transcripts examined were significantly stabilized in the absence of *rent1/hUpf1*, demonstrating that they are directly regulated by the nonsense surveillance machinery. These data provide the first systematic analysis of endogenous mammalian transcripts regulated by nonsense surveillance and dramatically expand the known repertoire of genes regulated by NMD. Our results document that nonsense surveillance constitutes a critical post-transcriptional regulatory mechanism that influences the expression of thousands of mammalian transcripts and the proteins they encode.

**Human L1 Transcription and Retrotransposition are Regulated by Transcription Factor RUNX3.** *N. Yang<sup>1</sup>, L. Zhang<sup>2</sup>, Y. Zhang<sup>1</sup>, H.H. Kazazian Jr.<sup>1</sup>.* 1) Department of Genetics, University of Pennsylvania, Philadelphia, PA; 2) Center for Research on Reproduction and Women's Health, University of Pennsylvania, Philadelphia, PA.

LINE-1s are abundant non-LTR retrotransposons that comprise 17% of the human genome. The 5' untranslated region (5'UTR) of human L1 (L1Hs) houses a poorly understood internal promoter. Here we report that mutations at a putative RUNX site (+83 to +101) in the 5'UTR decrease L1Hs transcription and retrotransposition up to 90% in cell culture-based assays. Exogenous expression of RUNX3, but not the other two RUNX family members, RUNX1 and RUNX2, increases L1Hs transcription (by 2-fold) and retrotransposition (by 70%), which are otherwise decreased by siRNAs targeting RUNX3 (up to 70%) and a dominant negative RUNX (up to 87%). Furthermore, the specific interaction between RUNX3 and its binding site is demonstrated by an electrophoretic mobility shift assay (EMSA) using an anti-RUNX3 antibody. Previously an antisense promoter has been shown in the L1Hs 5'UTR in the +400 to +600 region. However, no specific transcription factor or nucleotide sequence has been implicated. Here we show by site-directed mutagenesis and exogenous expression of RUNX factors that RUNX3 can regulate the antisense promoter activity of L1Hs 5'UTR via another putative RUNX site (+526 to +508). Our results indicate an important role for RUNX3 in L1Hs retrotransposition as well as transcription from its 5'UTR in both sense and antisense directions. They will contribute to our understanding of the mechanism underlying L1Hs retrotransposition and its impact on the expression of adjacent cellular genes.

**Expression of mutant TBP and huntingtin in cultured cells.** *M. Friedman*<sup>1,2</sup>, *SH. Li*<sup>2</sup>, *XJ. Li*<sup>2</sup>. 1) Graduate Program in Gen/Molec Biol, Whitehead, Emory Univ, Atlanta, GA; 2) Dept Human Genetics, Whitehead, Emory Univ, Atlanta, GA.

Considerable phenotypic similarity is observed among the nine known polyglutamine (polyQ) diseases with regard to clinical manifestation, pathology, and molecular changes. Transcriptional dysregulation, which can be induced by aberrant interactions between polyQ proteins and transcription factors, has been proposed as a potential basis for the pathogenesis of polyQ diseases. Previous studies suggest that the TATA-binding protein (TBP) is recruited into polyglutamine aggregates formed by mutant huntingtin (htt), a protein of unknown function that causes Huntingtons disease (HD). Expansion of a polyglutamine repeat (>44 repeats) in TBP causes neurological symptoms and neuropathology in spinocerebellar ataxia 17 (SCA17) with marked similarities to what is observed in HD. Thus, it is interesting to study whether mutant TBP forms inclusions that can recruit normal huntingtin. We have generated TBP cDNA constructs containing 15, 38, 60, and 98 CAGs. Expression of these cDNAs in HEK 293 cells indicated that only pathogenic repeats (60 and 98 CAGs) cause TBP to form small aggregates in the nucleus. When co-transfected with normal huntingtin, mutant TBP was still concentrated in the nucleus. No obvious co-localization of mutant TBP and huntingtin was observed. However, similar to mutant huntingtin transfected cells, HEK 293 cells expressing mutant TBP had reduced viability as compared with those expressing normal TBP. Expression of mutant exon1 huntingtin resulted in aggregates that were primarily localized in the cytoplasm. When normal TBP and mutant huntingtin were co-transfected into HEK 293 cells, the latter induced the formation of predominantly cytoplasmic aggregates while the former was detected almost exclusively in the nucleus. Although it remains possible that soluble mutant htt may interact with TBP, the different subcellular localization of mutant TBP and huntingtin suggests that they may mediate cytotoxicity in transfected cells via different mechanisms.

**Role of a Tetranucleotide Repeat Polymorphism in the First Intron of the Tyrosine Hydroxylase Gene in Expression and Behavioral Phenotypes.** *X. Hu*<sup>1</sup>, *C. Mazzanti*<sup>2</sup>, *R.H. Lipsky*<sup>1</sup>, *D. Goldman*<sup>1</sup>. 1) Laboratory of Neurogenetics, NIAAA, NIH, Rockville, MD; 2) NCI, NIH, Bethesda, MD.

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the biosynthesis of catecholamines that include dopamine, noradrenaline, and adrenaline. These catecholamines are important neurotransmitters and hormones that regulate visceral functions, motor coordination, and arousal and are highly expressed in certain neurons of the ventral tegmental area or the locus ceruleus. The TH gene is located on the short arm of chromosome 11 (11p15.5). A variable number tandem repeat (VNTR) composed of TCAT repeat units with seven alleles from 5-11 repeat units is located within intron 1. The TH10 allele is composed of perfect repeats (TH10p) and a variant (TH10i) that has a 1-bp deletion creating an incomplete repeat unit. An association of the 10p allele with schizophrenia was found in one population, supporting a possible link with dysregulated dopamine synthesis and the disease. The TH VNTR polymorphism influences transcriptional activity. When linked to a CMV promoter, we determined that allelic variations of HUMTH01 commonly found in humans had a quantitative silencing effect on TH gene expression. To determine if the allelic variants affected activation of the TH gene, we initiated a more detailed expression study using the native TH promoter as part of a "mini-gene" construct composed of exon1, intron1, and exon 2 fused to a DsRed reporter gene. Six constructs containing different alleles of the repeat were selected for expression studies in different cell lines under the control of the cAMP-responsive element within the TH promoter. Each of the six alleles allow for forskolin-dependent activation of the DsRed reporter. We are currently quantifying the response to determine the contribution of each allele to TH expression.

**Cloning and expression studies of zebrafish *pitx3* gene.** *D. Bosenko*<sup>1</sup>, *N. Zinkevich*<sup>1</sup>, *B. Link*<sup>2</sup>, *E.V. Semina*<sup>1</sup>. 1) Pediatrics, Medical College of Wisconsin, Milwaukee, WI; 2) Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI.

*PITX3* is a homeodomain transcription factor involved in anterior segment dysgenesis, cataracts and glaucoma in humans and *aphakia* in mice. We have now identified zebrafish *pitx3* gene and studied its expression and sequence. We used PCR primers designed from human and frog *pitx3* sequences to amplify different regions of the gene. The combined sequence of the zebrafish *pitx3* was found to be highly homologous to the human gene with overall 67% identity at the nucleotide and 62% at the protein level. The HD region showed 100%, the C-terminal region 70% and the N-terminus 65% identity. Zebrafish *pitx3* and *pitx2* proteins were found to be 67% identical to each other with most identity seen in the HD (100%), then C-terminal (78%) and divergent N-terminal regions (10%). Expression of zebrafish *pitx3* was studied by *in situ* hybridization with dig-labeled antisense riboprobe in 18-20, 24 and 48-hour embryos. The first expression was detected in the forebrain and midbrain regions in 18-h embryos continuing at least till 48-h stage. The first ocular expression of *pitx3* was evident in 19-20-h embryos at the stage of lens placode. The *pitx3* transcripts are abundant in the eye region in 24-h embryos at the lens vesicle stage. In the 48-h embryos, the expression was detected in the equatorial and posterior regions of the lens and in the periocular mesenchyme. The expression pattern of zebrafish *pitx3* gene is highly conserved with other species and therefore zebrafish represents a good model to study regulation of *pitx3* and its molecular pathways, aberrations of *pitx3* may result in lens phenotypes. Therefore we are studying *pitx3* sequence for mutations in a69 zebrafish mutant. The phenotype of the a69 mutant is characterized by arrest of lens development. Further studies of *pitx3* expression in the a69 mutant and wt fish are ongoing. We have also identified partial genomic sequence of the zebrafish *pitx3* gene and located several regions of conservation outside of *pitx3* coding sequence. These regions are likely to play role in regulation of *PITX3* expression and are being investigated further.

**Identification and characterization of regions involved in regulation of Pitx2 expression.** N. Zinkevich<sup>1</sup>, D. Bosenko<sup>1</sup>, K. Frees<sup>2</sup>, M. Shi<sup>2</sup>, Y. Trembath<sup>2</sup>, J.C. Murray<sup>2</sup>, B. Link<sup>3</sup>, E.V. Semina<sup>1</sup>. 1) Pediatrics, Medical College of Wisconsin, Milwaukee, WI; 2) Pediatrics, University of Iowa, Iowa City, IA; 3) Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI.

*PITX2* is a homeodomain transcription factor associated with Axenfeld-Rieger syndrome in humans and multiple animal phenotypes identified by misexpression and knockout studies. Therefore, precise level, site and timing of *PITX2* expression are important for normal development. In order to reveal elements involved in regulation of *PITX2* we first searched for regions of sequence conservation outside of *PITX2* coding region using human, mouse, rat, fugu and zebrafish *Pitx2* genomic sequences identified by a combination of database searches and our own sequencing efforts. *PITX2* gene was found to span ~ 20 kb in human, mouse and rat genomes, ~ 9kb in fugu and ~ 15 kb in zebrafish genomic sequence. Human *PITX2* sequence including 100-kb of 5' and 20-kb of 3' sequence was compared to other sequences and ten highly conserved regions have been identified. Five elements are located 5' of *PITX2*, four are internal regions but located upstream of *PITX2c* isoform and one internal element. The average length of the conserved sequence is ~ 150 nt and average identity ~ 76%. We hypothesized that these regions are enhancers involved in regulation of *PITX2*. We plan to use zebrafish transgenic to identify specific sites and timing of expression controlled by these elements. Down this path, we first analyzed expression of zebrafish *pitx2*. The first expression was detected at 90% epiboly stage (9 hrs) as reported by others. In 48-h embryos, a strong signal was seen in discrete regions in the brain, periocular mesenchyme, brachial arches and heart with expression in these regions continuing at least till 72-h stage. This expression pattern is conserved with other species and therefore zebrafish represents a useful model for identification of elements involved in regulation of *PITX2*. The conserved regions have been cloned into GFP-reporter plasmids and are now being tested for expression in zebrafish transient transgenics.

**Mig12, a novel Opitz syndrome gene product partner, co-operates with Mid1 to stabilize microtubules.** *B. Fontanella, C. Berti, R. Ferrentino, A. Ballabio, G. Meroni.* TIGEM, Napoli, Italy.

Opitz G/BBB syndrome (OS) is a genetic disorder characterized by developmental midline abnormalities, such as hypertelorism, cleft of lip and palate, and hypospadias. The gene responsible for the X-linked form of this disease, MID1, encodes a TRIM/RBCC protein that is anchored to the microtubules. The association of Mid1 to the cytoskeleton is regulated by dynamic phosphorylation, through the interaction with the 4 subunit of phosphatase 2A (PP2A). This interaction with 4 also involves Mid1, acting as an ubiquitin E3 ligase, in the control of PP2A degradation.

In spite of these data, the biological role exerted by the OS gene product is still to be elucidated and the presence of other potential interacting moieties in Mid1 structure prompted us to search for additional cellular partners through a yeast two-hybrid screening. We identified a novel gene, MIG12, whose protein product interacts with Mid1. Transiently expressed Mig12 is diffused in both nucleus and cytoplasm, although enriched in the microtubule-organizing center (MTOC) region. When co-expressed with Mid1, Mig12 is massively recruited to filamentous structures composed of tubulin. These bundles are resistant to high dose of microtubule depolymerizing agents and are composed of acetylated tubulin, thus representing stabilized microtubule arrays. Microtubule stabilization is crucial in processes such as cell division and migration. Mig12 high expression in the embryonic neuroepithelial midline and urogenital apparatus suggests that Mid1-Mig12 complexes might be implicated in such cellular processes during the development of some of the midline systems that are affected in OS patients.

**Dystrophin Dp71 isoforms expressed in PC12 cells. Interaction between Dp71f and DGC.** *C. Montanez<sup>1</sup>, J. Romo-Yanez<sup>1</sup>, F. Depardon<sup>1</sup>, V. Ceja<sup>1</sup>, A. Rendon<sup>2</sup>, D. Mornet<sup>3</sup>, F. Velazquez<sup>1</sup>*. 1) Dept Genetics & Molec Biol, CINVESTAV IPN, Mexico, Mexico; 2) Laboratoire de Physiopathologie Cellulaire et Moleculaire de la Retine, Hospital Saint-Antoine, Paris, France; 3) Institute National de la Sante et de la Recherche Medicale, U 128 IFR, Montpellier, France.

Duchenne Muscular Dystrophy (DMD) is a lethal X chromosome-linked recessive disease. This disorder is caused by mutations in the DMD gene that encodes dystrophin. A 71kDa C-terminal dystrophin isoform (Dp71) has been identified as the major product in brain. It has been suggested that Dp71 is involved in the anchorage and/or organization of specific membrane components. It is also known that Dp71mRNA goes through alternative splicing. Our group is interested in studying Dp71 function in nervous system, for this reason, we have examined the expression of Dp71 isoforms in PC12 cell line and we have shown that the expression and localization of two Dp71 isoforms is differentially regulated during PC12 cells NGF-induced differentiation. To gain insight into the function of each Dp71 isoform, we isolated, three cDNA clones corresponding to Dp71 isoforms expressed in PC12 cells. One cDNA clone corresponds to the isoform lacking exon 71, the second cDNA clone corresponds to the transcript lacking exons 71 and 78. Interestingly, a third cDNA clone encodes a novel and low abundant Dp71 isoform lacking exons 71 to 74. Sequence analysis shows that these cDNAs are highly homologous to human Dp71 isoforms. With the aim of identify proteins of the DGC that interact with Dp71 isoforms, we first analyzed DGC proteins expression in PC12 cells by Western blot. The results show that PC12 cells express -dystroglycan, 1-syntrophin, 1-dystrobrevin and -dystrobrevin proteins. We have established by co-immunoprecipitation assays that Dp71f forms a complex with -dystroglycan, 1-syntrophin, and -dystrobrevin in PC12 cells which strongly supports the existence of a DGC-Dp71f structural and/or functional complex in PC12 cells. We will use Xpress and/or Myc-tagged versions of each Dp71 isoform to farther identify interacting proteins. This work was supported by Conacyt 37515.

**Developing an Illustrated Context-Sensitive Medical Genetics Glossary.** *R. Pagon<sup>1</sup>, C. Abair<sup>1</sup>, L. Ribas<sup>1</sup>, J. Edwards<sup>1,2</sup>, A. Amemiya<sup>2</sup>, G. Feldman<sup>3</sup>, M. Espeseth<sup>1</sup>, N. Stevens<sup>1</sup>, J. Breuner<sup>4</sup>, L. Pinsky<sup>1</sup>, K. Marymee<sup>1</sup>, C. Dolan<sup>1,2</sup>, P. Baskin<sup>1</sup>, P. Tarczy-Hornoch<sup>1</sup>.* 1) U of WA, Seattle; 2) Children's Hosp and Reg Medical Ctr, Seattle; 3) Wayne State U, Detroit; 4) Swedish Hosp, Seattle.

**SIGNIFICANCE:** The GeneTests (www.genetests.org) Laboratory Directory information on medical genetics testing and the GeneReviews descriptions of test use need to be useful to both genetic and non-genetic healthcare professionals.

**BACKGROUND:** GeneTests uses a specialized vocabulary, yet the majority of its 20,000 searches/day are by non-geneticists. Most genetics resources for clinicians do not explain molecular genetic test methods used by clinical labs.

**METHODS:** The GeneTests staff and three primary care physicians developed an illustrated context-sensitive glossary. Over 220 terms on the GeneTests Web site most relevant to genetic testing and genetic counseling were defined using textbooks and other glossaries as references. Over 100 terms were illustrated with a Learn More page, a section on Some Clinical Implications, and either a Case Example vignette or a Disease Example discussion. Context-sensitive links to glossary definitions appear in the Methodology section of the Laboratory Directory and in all sections of each GeneReview, where the reader can simultaneously view the relevant definition and text.

**RESULTS:** 1) Primary care physicians requested that a) the glossary, which defines genetic counseling and testing terms, be accompanied by educational materials that elaborate on the same concepts, b) glossary terms be categorized and case examples created for teaching purposes. 2) In April 2003, glossary entries were viewed ~200 times/day and the full glossary ~150 times/day. About 25% of users chose to leave the GeneReviews glossary links enabled.

**CONCLUSIONS:** 1) Both clinical geneticists and non-genetic healthcare professionals need an accessible glossary that includes definitions of molecular genetic test methods. 2) For effective communication among geneticists and between geneticists and non-geneticists, shared meaning of terms is necessary.

**Transmission of Full Mutation Alleles from Premutation Males to Daughters.** *W.T. Brown<sup>1</sup>, S.L. Nolin<sup>1</sup>, C. Dobkin<sup>1</sup>, L. Gane<sup>2</sup>, X.D. Ding<sup>1</sup>, G.E. Houck<sup>1</sup>, A.D. Gargano<sup>1</sup>, A. Glicksman<sup>1</sup>, S.Y. Li<sup>1</sup>.* 1) Dept Human Genetics, NYS Inst Basic Research, Staten Island, NY; 2) UC Davis M.I.N.D. Institute, Sacramento, CA.

In Fragile X, the dramatic CGG expansion to >200 repeats in a full mutation occurs nearly exclusively from premutation females to their offspring. Males with premutations or full mutations have premutation-size repeats in sperm and transmit premutations to their daughters. We observed two unrelated transmissions of full mutations from premutation males to their daughters. In the first family, the CGG repeat was examined by PCR in the mother, father and three daughters. The mother was found not to be a carrier. The father had 105 repeats and two of the daughters had 100 and 95 repeats. The third daughter had predominantly 145 repeats and a series of bands extending into the full mutation range. Southern analysis revealed a series of unmethylated fragments extending from premutation to full mutation, and the normal allele. In the second family, PCR and Southern analysis of one daughter of a premutation male revealed repeats from 135 to >200. The unusual molecular patterns of these two females were notable for the exclusively unmethylated fragments; a pattern not associated with females carrying maternally transmitted full mutation alleles. Two similar cases have been reported (Bridge, *Gen Med*, 23A, 1999; Ventura, *AJHG*, A471, 1999). We suggest a paternally transmitted allele may expand to a full mutation with 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 lead to aberrant methylation. We suggest paternal transmission of full mutations involves unique mechanisms.

**Assessment of patient response to genetic counseling for hereditary pancreas cancer.** *J.E. Axilbund, K.A. Brune, M.I. Canto, B.C. Brehon, L.D. Wroblewski, C.A. Griffin.* The Johns Hopkins University, Baltimore, MD.

**Introduction:** Genetic counseling is currently offered for a variety of cancers, including breast, ovarian and colorectal cancers. Genes have been discovered that cause a significant percentage of these cancers, and genetic testing is available. Although pancreas cancer is associated with several known hereditary syndromes, a major causative gene for this cancer remains undiscovered. It is unknown if genetic counseling in the absence of a known gene is beneficial to patients. **Methods:** At-risk patients with three or more family members with pancreas cancer received genetic counseling while participating in an endoscopic ultrasound screening study. Participants were mailed a questionnaire after the visit regarding their views about the genetic counseling session. Responses were based on a 5-point Likert scale, ranging from "strongly agree" to "strongly disagree". **Results:** Thirty-two participants responded, having an average of 3.75 relatives with pancreas cancer. 69% completed college or graduate school, and 69% had read information about hereditary pancreas cancer. 84% agreed that the genetic counseling session was helpful, and 88% would recommend genetic counseling for pancreas cancer to a friend or relative with a family history of the disease. 94% "agreed" that genetic counseling for pancreas cancer is helpful despite the lack of an identified major causative gene, and 88% "disagreed" that genetic counseling should not be offered until such a gene is identified. 84% would want to be tested if a major causative gene were found, and 91% would be interested in another genetic counseling session when more information is learned. Respondents were ambivalent about the ability of currently available genetic testing to help them, as well as their desires to pursue such testing. However, 66% felt that the pancreas cancer in their family was caused by a gene mutation, and 44% thought that they had inherited the mutation. **Conclusions:** Despite the lack of an identified major causative gene for pancreas cancer, the majority of patients at increased risk find genetic counseling for this disorder to be helpful. Supported by NCI grant CA78148-05.

**Effect of Genetic Counseling for Hereditary Breast and Ovarian Cancer (BOC) on Health Behaviors and Perceived Personal Control (PPC).** *S. Hensley-Alford*<sup>1</sup>, *R.R. Lebel*<sup>1, 2</sup>, *R. Pollack*<sup>1</sup>, *J.R. Roberson*<sup>1</sup>, *D.L. Van Dyke*<sup>1</sup>. 1) Henry Ford Hospital, Detroit, MI; 2) Hinsdale Hospital, Hinsdale, IL.

PPC and health behavior change have been studied in the context of genetic counseling for BOC risk. We examined self-reported changes in a population-based retrospective cohort of 218 women counselled for BOC between 1/1/00 and 2/28/03. We sent questionnaires twice, 2-4 weeks apart; second mailing was followed by a phone call within 4 weeks. Mean age of respondents 50; 88% white, 88% married or living with a partner, 85% with children, 43% with BOC, 85% had some college, 66% had household income of \$80,000 or more. We requested reports on general health behaviors (diet, exercise, check-ups, vitamin use, prescription drug use, smoking) and breast health behaviors (mammography, clinical and self- breast exam, chemoprevention, prophylactic surgery) before and after genetic counseling. Using a Wilcoxon Signed Rank test, we evaluated reported behavior changes. 47% of women reported change in at least 1 general health behavior, with significant changes for diet ( $p=0.035$ ), exercise ( $p=0.002$ ), multivitamin use ( $p<0.001$ ). 41% of women reported change in at least 1 breast health behavior. There was marginal evidence in for chemoprevention ( $p=0.065$ ) and prophylactic surgery ( $p=0.070$ ). We asked a standard set of questions for PPC. 68% of women reported change in at least 1; the importance of family history ( $p=0.005$ ), effect of cancer inheritance ( $p<0.001$ ), BOC risk ( $p<0.001$ ), evaluating risk management options ( $p<0.001$ ), making decisions about familys future regarding breast cancer ( $p=0.003$ ), easing the situation regarding breast cancer ( $p<0.001$ ), deciding next steps regarding BOC risk ( $p<0.001$ ). Our data suggest that genetic counseling for BOC may impact health behaviors, with effects stronger for general health than breast health. The women participating in this study are not representative of the general population, suggesting that either the effects are limited to a subgroup receiving counseling or that other women may not elect genetic counseling or genetic counseling research. We find that the participants were representative of the population who are seeing us for counseling.

**Phenotypic heterogeneity in patients with Gaucher disease and the N370S (1226G) / V394L (1297T) genotype.** A. Zimran<sup>1</sup>, C.R. Scott<sup>3</sup>, M. Zeigler<sup>2</sup>, A. Abrahamov<sup>1</sup>, D. Elstein<sup>2</sup>. 1) Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel; 2) Genetics Laboratory, Hadassah Medical Center, Jerusalem, Israel; 3) Molecular Diagnostic Laboratory, Childrens Hospital & Regional Medical Center, Seattle WA; USA.

Correlation between genotype and phenotype in Gaucher disease is limited. It is known that the most common mutation N370S (1226G) is protective of or at least mitigates neurological involvement, but for the V394L (1297T) mutation, described as the fifth most common among Ashkenazi Jews, in whom there is a predilection for Gaucher disease, little data is available. Too, the V394L mutation is included in standard kits for large-scale screening. This study reports all known patients with Gaucher disease who are documented to have the N370S/V394L genotype from a large referral clinic in Israel and from the International Collaborative Gaucher Group (ICGG) registry. Of 476 patients in the Jerusalem Gaucher Clinic, seven patients (2.0%) had the N370S/V394L genotype; of 2836 patients in the registry there were 14 patients (0.8%) with this genotype. There was an overlap of three patients making a total of 18 patients, reflecting the rarity of this genotype among the studied cohorts. Most of these patients had mild disease: while eight of these patients required specific enzyme therapy, none was splenectomized and only three patients had skeletal involvement, but other baseline parameters were very diverse. Although genotype-phenotype correlation in this case may be difficult because of the low numbers, since the V394L mutation when seen in a compound heterozygote with a null allele results in neuronopathic disease, one cannot conclude that this mutation is protective of neuronopathic disease in a manner similar to the N370S mutation, and hence may have continued importance for large-scale screening of at risk populations.

**Posttraumatic stress disorder of women after stillbirth with fetal anomalies.** *S. Sohda*<sup>1, 2</sup>, *H. Tsukamoto*<sup>2</sup>, *Y. Fujiki*<sup>2</sup>. 1) Dept OB/GYN, Mito Saiseikai General Hospita, Mito, Japan; 2) Dept OB/GYN, University of Tsukuba, Tsukuba, Japan.

At least 15 percent of clinically recognized pregnancies result in miscarriage during the first trimester. Stillbirth with fetal anomalies occurs suddenly in the second and third trimester. Posttraumatic stress disorder (PTSD) has been found after pregnancy loss in the first trimester and some pregnancy complications. However PTSD has not been assessed in women who have had stillbirth with fetal anomalies. We studied about the mental health of women who have had stillbirth due to congenital fetal anomalies included in the cases with prenatal diagnosis. To assess PTSD, we used the Impact of Event Scale-Revise (IES-R). The IES-R can be a useful self-rating diagnostic instrument particularly for survivors with PTSD symptoms as a clinical concern by using a 24/25 cutoff in total score. Of the 11 women who had a stillbirth, the score of the IES-R is 26.713.3(averageSD). Over 50% of women who had a stillbirth were PTSD, 5 (46%) women were over 30 with score of IES-R. Our result suggests we have to make same psychosocial care system and educational system for women who had stillbirth. It is need that further debate and research in the area.

**Who should be tested? Genetic counselors' opinions of carrier testing.** *C.L. Cina, S. Metzenberg, A. Metzenberg, M. D'Addario.* California State University, Northridge, Northridge, CA.

During a prenatal genetic counseling session, many clients are now routinely given the option of carrier testing, even without an indication or a family history. Due to the growing number of carrier tests available, genetic counselors are often devoting more of their time to discuss testing options. The purpose of this study was to investigate what types of carrier testing are routinely offered to prenatal clients, and to assess the opinions of genetic counselors towards carrier testing by determining what factors they feel are important when offering testing. The hypothesis was that the severity of a disorder, its frequency in an ethnic group, and the sensitivity of a test are the primary factors that counselors consider before offering carrier testing.

This study was conducted through an anonymous survey distributed at the National Society of Genetic Counselors Annual Education Conference and posted through a link from the NSGC website. A three-part questionnaire was designed to assess counselor demographics, opinions of carrier testing, and thoughts when presented with hypothetical and actual scenarios. A total of 371 responses was obtained.

The main findings were (1) genetic counselors are divided on what factors to consider before offering testing, (2) based on hypothetical and actual scenarios, a significant number of counselors favors broad testing, and (3) many counselors do not agree with their center policy regarding who should be tested. Comments provided by many respondents indicated that carrier testing is an individualized process that should be considered on a client-by-client basis. Based on the finding that counselors are divided on what to consider before offering testing, the service that clients receive may depend on counselors opinions. In addition, since a significant number of counselors favors routine testing, the implications of their beliefs on the health care system may need to be examined in light of the expected increase in availability of carrier testing.

Program Nr: 1098 from 2003 ASHG Annual Meeting

**A Comparison of Teaching Methods in Conveying Genetic Information.** *C.T. Dvorak, K.E. Jackson, H.C. Andersson, J.G. Thoene.* Hayward Genetics Center, Tulane University Medical School, New Orleans, LA.

Access to sources of reliable information about rare diseases is difficult in many parts of the country, particularly in rural areas. We assessed learning about lysosomal storage diseases in health care providers (nurses, physicians) and families of affected patients after a scripted teaching session, or after exposure to a video which covered the same material. The subjects took the same examination (10 questions, multiple choice) before and after the intervention. There were 21 subjects in each group. The base-line level of knowledge of lysosomal storage disorders was similar in both groups: 5.12.0 (video exposure) versus 4.71.6, (instructor exposure)  $p>0.4$ . After intervention a substantial improvement in knowledge was found in each group. The group exposed to the video scored 8.51.4 post exposure, compared to 5.12.0 pre-exposure ( $p=.005$ ) The group that experienced live instruction scored 8.11.2 post teaching, versus 4.71.6 pre-teaching ( $p=.075$ ). There was no significant difference between the post intervention scores (8.51.4 versus 8.11.2,  $p=0.4$ ). We conclude that, for general information unrelated to specific patient family circumstances, the use of educational videos can be an effective way to inform health professionals and families who do not have ready access to genetic professionals.

**Too much, too soon? Genetic testing offered at the same time as a breast cancer diagnosis in women under forty years of age.** *A. Ardern-Jones<sup>1</sup>, R. Kenen<sup>2</sup>, R. Eeles<sup>3</sup>.* 1) Dept Cancer Genetics, Royal Marsden NHS Trust, Sutton Surrey, United Kingdom; 2) The College of New Jersey, Ewing NJ, 08628 US; 3) Institute of Cancer Research and The Royal Marsden NHS Trust. Downs Road. Sutton. Surrey. SM2 5PT. UK.

Recent research suggests that women who develop breast cancer between the ages of 30-34 and have specific tumour characteristics e.g. high grade, oestrogen receptor negative type tumours have between a 10%-27% chance of being a BRCA1 gene carrier. Furthermore, recent research indicates that prophylactic mastectomy and/or oophorectomy offer a significant risk reduction in the development of breast/ovarian cancer. In the near future, patients may be offered the choice of a genetic test close to the time of diagnosis. This timing not only provides additional dimensions to treatment decisions, but has psycho-social familial implications as well. This small exploratory study investigates 1) whether or not women diagnosed with breast cancer under the age of 40 would want to be offered information about genetic testing close to the time of their diagnosis 2) whether the health professionals treating them support the idea 3) reasons for patients' and professionals' perspectives and concerns. We held focus groups of 13 women who had their only, or first, breast cancer under the age of 40 and who were known carriers of a BRCA gene how they felt about the timing. We also interviewed 14 health care professionals involved in various aspects of breast cancer care and cancer genetics. The large majority of former breast cancer patients and professionals believed that there was already emotional overload in coping with the cancer diagnosis and that adding genetic testing discussions would be too much. Some members of both groups, however, thought that offering genetic testing close to the time of breast cancer diagnosis might make sense because the results could alter treatment decisions.

Program Nr: 1100 from 2003 ASHG Annual Meeting

**Generalized Bayesian risk calculations for autosomal recessive disease when only one mutation is detected, incorporating test sensitivity and each parent's ethnicity information: what if a fetus has echogenic bowel and one detectable CFTR mutation?** *W. Grody*<sup>1</sup>, *S. Ogino*<sup>2, 3, 4</sup>. 1) Med Genetics/Molec Pathology, UCLA Sch Medicine, Los Angeles, CA; 2) Dept. of Pathology, Brigham and Women's Hospital, Boston, MA; 3) Dept. of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; 4) Harvard Medical School, Boston, MA.

Bayesian analysis plays a central role in risk calculations for genetic counseling. It is occasionally less than straightforward to calculate the risk for an autosomal recessive (AR) disease, especially when a fetus has one detectable mutation, and the frequencies of the disease allele and each mutation vary among various populations. A typical example is a fetus with echogenic bowel and one detectable CFTR mutation. We have developed novel generalized Bayesian methods to calculate the AR disease risk of a fetus when only one mutation is detected and another independent risk factor is present. Our methods incorporate such information as genetic test results on either or both of the parents, each parents ethnicity, and test sensitivity for the particular ethnicity. Excel spreadsheets to facilitate risk calculations are available upon request. We also meta-analyzed and estimated the conditional probabilities of echogenic bowel if the fetus has CF or not, as 0.55 or 0.0069, respectively. Using our methods, for example, if a fetus with echogenic bowel has one detectable CFTR mutation, and both parents (one northern and one southern Europeans without a family history of CF) have not been tested, the posterior disease risk of the fetus is ~0.22. In conclusion, our generalized Bayesian methods are versatile, and allow the AR disease probability to be calculated accurately, taking into account all relevant information.

**A Pilot Study of First-Degree Relatives of Patients with Colorectal Cancer Who Have Received Hereditary Cancer Education: Applying Family Hardiness and Health Belief Variables to Predict the Uptake of**

**Colonoscopy.** *P. Gambol<sup>1</sup>, V. Lagos<sup>1</sup>, G. Uman<sup>2</sup>, L. Jacobs<sup>3</sup>, M. Grant<sup>1</sup>, D. MacDonald<sup>1</sup>, J. Weitzel<sup>1</sup>.* 1) Clinical Cancer Genetics, City of Hope, Duarte, CA; 2) Vital Research, LLC, Los Angeles, CA; 3) University of Pennsylvania Cancer Ctr, Ardmore, PA.

The risk for colorectal cancer (CRC) ranges from 5% to 80% depending on age, family history, and genotype. An individual with 2 affected first-degree relatives (FDRs) has a 17% risk for developing CRC; mortality rates are directly proportional to cancer stage at diagnosis. Decreased mortality is achieved by early detection. Our first objective was to measure Family Hardiness and Health Beliefs to predict the uptake of colonoscopy in FDRs of persons with CRC. Our second was to determine if hereditary cancer education given to persons with CRC increases the uptake of colonoscopy in their FDRs. Methods: A case-control study of 2 distinct cohorts was conducted. Cases: Persons with CRC that have received hereditary cancer education and their FDRs. Historical Controls: Persons with CRC that have not received hereditary cancer education and their FDRs. Persons with CRC (N=30) were recruited, 14 males and 16 females with a mean age at diagnosis of 41. Fifty percent completed surveys that measured communication and Family Hardiness. Fourteen FDRs, 4 males and 10 females with a mean age of 46 completed a demographic and a Health Belief survey. Results: FDRs of cases were White (57%), Hispanic (36%) and African American (7%) with no prior cancer diagnosis. Fifty seven percent had a college degree and 21% had less than a high school education. Eleven (79%) were told by their family member that they were at an increased risk for CRC. Colon screening was undertaken by 71% (57% had a colonoscopy and 14% had a sigmoidoscopy) compared to 41% in the historical controls. Logistic regression analysis revealed that two Health Belief Model variables (benefits and barriers) were predictive of FDRs participation in lower GI endoscopy. Conclusion: FDRs of cases had a higher uptake of lower GI endoscopy (20% higher) than controls. In addition FDRs of cases with less than a high school education had a high uptake of colonoscopy (100%).

**Cancer Surveillance and Preventative Surgery in Members of BRCA1/2 Positive Families.** *S. Nanda<sup>1</sup>, G. Sheridan<sup>1</sup>, N. Scanlan<sup>2</sup>, A. Horler<sup>1</sup>, J.H. Jung<sup>1,2</sup>, P.J. Ainsworth<sup>1,2</sup>.* 1) London Health Sciences Centre, Ontario, Canada; 2) London Regional Cancer Centre, Ontario, Canada.

Individuals with germline BRCA1/2 mutations are known to be at increased lifetime risk of developing breast, ovarian and prostate cancer. Clinical genetic testing for BRCA1/2 is available however, little information is available on the impact of genetic testing on an individual's life. This study aims to assess the impact of genetic testing on screening for breast/ovarian cancer and uptake of preventative surgery in members of families with a known BRCA1 or BRCA2 mutation. A questionnaire assessing participation in cancer screening and uptake of preventative surgery was sent to 203 individuals from known BRCA1/2 families in Southwestern Ontario. All individuals had received their results at least 6 months prior to receiving the questionnaire. Of the participants, 84.3% were female, 54.9% were mutation carriers and 45.1% were non-carriers. Twenty-eight percent were diagnosed with breast cancer and 5.9% were diagnosed with ovarian cancer. Of carriers (male and female), 51.8% had not made changes to their cancer surveillance practices since receiving their test results. Of the female carriers, 41.3% proceeded with prophylactic mastectomy (PM) and 58.7% proceeded with prophylactic oophorectomy (PO) after receiving their test results. Of the non-carriers (male and female), 69.6% had not made changes to their cancer surveillance practices since receiving their test results. None of the female non-carriers proceeded with prophylactic surgery after receiving their test results, however 27.3% were still considering PM and 40% were still considering PO. Although not statistically significant, women under the age of 50 and participants with a high school education or higher were more likely to consider prophylactic surgery. Overall, a majority of participants did not alter their screening practices based on their test results though a substantial proportion of carriers did opt for prophylactic surgery. Interestingly, many non-carriers were still considering prophylactic surgery.

Program Nr: 1103 from 2003 ASHG Annual Meeting

**Use of video for patient education: A survey of cancer genetic counselors.** *L.A. Hamby, J.E. Axilbund, D.B. Thompson, S.J. Olsen, C.A. Griffin.* Mid-Atlantic Cancer Genetics Network, The Johns Hopkins University, Baltimore, MD.

Cancer genetic counselors use a variety of teaching modalities for patient education. The prevalence of video use in cancer genetic counseling is unclear, and little is known about genetic counselors' preferences regarding its use. Fifty cancer genetic counselors responded to a survey assessing their use of educational videos and their recommendations for content of future videos. Thirty percent (n=15) of respondents currently use videos for patient education. The most commonly cited benefits included reinforcement of information (n=9), ability to share information with other family members (n=7), and increased counselor efficiency (n=6). Of the 70% (n=35) who do not use videos, predominant barriers included the perceived lack of an appropriate or unbiased video (n=14), lack of space and/or AV equipment (n=11), and concern that videos are impersonal (n=10). If an appropriate video were developed, 69% of respondents would be "likely" or "somewhat likely" to use it before the genetic counseling visit, while 83% gave a similar response for after the visit. Additional suggestions included use with referring physicians and for professional education. Regarding content, respondents desired a video that is inclusive and representative of the genetic counseling session, but emphasized the importance of using broadly based information. All respondents ranked the following content areas as "critical" or "somewhat important": the pros and cons of genetic testing, psychosocial implications, and genetic discrimination. The next most desired content areas included a description of inheritance, the basis of DNA and chromosomes, and cancer development. Ranked by the majority of respondents as "not at all important" was information about the human genome project. This exploratory study provides data relevant for development of an educational video to be used in association with a personalized genetic counseling session. Supported by NCI grant CA78148-05.

**SUCCESSSES AND CHALLENGES IN GENETICS EDUCATION OF HEALTH PROFESSIONALS: THE HUMAN GENOME EDUCATION MODEL II (HuGEM II) PROJECT EXPERIENCE.** *C. Kozma<sup>1</sup>, EV.*

*Lapham<sup>1</sup>, C. Dunne<sup>1</sup>, JO. Weiss<sup>2</sup>, MA. Wilson<sup>2</sup>, J. Benkendorf<sup>1</sup>.* 1) Department of Pediatrics , Georgetown University Hospital, Washington, DC; 2) The Genetic Alliance, Washington, DC.

After completing four years of educating health professionals across the country as part of the HuGEM II Project, analysis of the education methods provides important information. The HuGEM II Project, a collaborative effort between Georgetown University and the Genetic Alliance (1997-2001, NIH funding) used a three-pronged approach to the genetics education of the members of seven national health professional organizations. The faculty was multidisciplinary and composed of health professionals as well as consumers. The focus of the education included the Human Genome Project, its related Ethical, Legal, and Social issues, and the impact of genetics progress on the perspective discipline. Three distinct groups within each organization, Administrators, Leader Educators and Practitioners, were targeted for genetics education, each with a unique educational approach. 1) Orientation sessions averaging 1 hour were held with 130 national staff and board members of the associations. 2) A week-long core course in genetics was attended by 60 health professional Leaders Educators. 3) Training workshops averaging 3 hours, were held at 48 national and regional conferences of the associations for over 1550 health professional practitioners. Results of the evaluation data for each approach will be presented. The HuGEM II Project provides a replicable model for working with health professionals organizations in a collaborative genetics education mode.

**Genetic counseling of medullary breast cancer patients.** *C. Kroupis*<sup>1</sup>, *N. Goutas*<sup>2</sup>, *S. Vasilaros*<sup>3</sup>, *D. Yannoukakos*<sup>4</sup>, *E. Lianidou*<sup>1</sup>, *M.B. Petersen*<sup>5</sup>. 1) Laboratory of Analytical Chemistry, Dept. of Chemistry, University of Athens, Athens, GREECE; 2) Pathology Lab, Evgenidion Hospital, Athens, GREECE; 3) Prolipsis Breast Cancer Center, Athens, GREECE; 4) Molecular Diagnostics lab, Demokritos Research Center, Athens, GREECE; 5) Dept. of Genetics, Institute of Child Health, Athens, GREECE.

Medullary carcinoma of the breast is very rare (<3% of cases in most populations). An overrepresentation of these carcinomas among patients with mutations in the BRCA1 gene has recently been reported and it has been proposed that all breast medullary cancer patients should be referred for BRCA1 mutation analysis. The goal of our study was to examine the validity of such a proposal in 17 patients with this rare carcinoma in Greece. **METHODS.** Patients were asked about their family history in counseling sessions. After informed consent, mutation analysis was performed on genomic DNA isolated from blood leukocytes employing a combination of the Protein Truncation Test (PTT) for the large exon 11 of the BRCA1 gene and DNA sequencing of the other small exons. Various techniques were performed in order to assess phenotypic characteristics in the corresponding tumor specimens: immunohistochemistry (IHC) for the presence of hormone receptors, Ki-67 proliferation marker, accumulation of p53 protein and overexpression of c-erbB-2 and cytometry for DNA ploidy and S-phase estimation. **RESULTS.** Only five patients had a positive family history. Seven out of the 17 tumor specimens were showing the phenotype of a BRCA1 carcinoma: negative for hormone receptors, overexpression of c-erbB-2, and also highly proliferative as seen by the intense Ki-67 immunostaining, high S-phase fraction. Mutations were detected in 2 patients: the recurrent in the Greek population 5382insC mutation in exon 20 and the rare R1203X in exon 11 in patients whose tumors fulfilled the phenotypic criteria of a BRCA1 carcinoma, but only one of the patients had a family history. **CONCLUSION.** Breast medullary carcinoma patients should be referred for the expensive BRCA1 mutation analysis only when they possess either family history or the appropriate phenotypic characteristics in their tumors.

**Accurate carrier risk calculations for autosomal recessive disorders.** *R. Wilson*<sup>1</sup>, *S. Ogino*<sup>2, 3, 4</sup>. 1) Pathology/ Lab Med, Rm 509A, Univ Pennsylvania, Philadelphia, PA; 2) Pathology, Brigham and Women's Hospital, Boston, MA; 3) Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; 4) Harvard Medical School, Boston, MA.

**Introduction:** Risk assessment is an essential component of genetic counseling and testing. Carrier risks for autosomal recessive disorders can be calculated using Bayesian analysis, taking into account family history and/or genetic test results. However, it is often less than straightforward to calculate the carrier risks of relatives distant from the index case of an autosomal recessive disorder with a known carrier frequency. Non-negligible de novo mutation rates further complicate the calculation of carrier risks in such scenarios. **Methods:** We developed novel systematic Bayesian methods to calculate carrier risks for autosomal recessive disorders when the disease allele frequency or de novo mutation rate is non-negligible. Excel spreadsheets that can be used independently or sequentially to calculate carrier risks in various typical settings are available upon request. **Results:** The effect of the disease allele frequency on the carrier risk is larger when the relationship to the affected is more distant. Using our methods, e.g., the carrier risk of a second cousin of a patient with cystic fibrosis is calculated as ~0.0900 (rather than 0.0625), taking into account the relatively high disease allele frequency for cystic fibrosis. The number of unaffected siblings has little effect on risk calculations. The risk that the fetus of a consanguineous couple is affected depends not only on the carrier risks of the parents, but also on whether the mother's carrier probability is independent of the father's. The probabilities that the fetus is affected with two mutations that are identical-by-descent, or identical-by-state, need to be considered. **Conclusion:** Our generalized Bayesian methods are versatile and allow accurate carrier risk assessments for autosomal recessive disorders, taking into account all relevant information.

**Genetic Counselling and Testing Process in BRCA1/2 Families- Are Patients Satisfied?** *N. Scanlan<sup>1</sup>, G. Sheridan<sup>2</sup>, S. Nanda<sup>2</sup>, A. Horler<sup>2</sup>, J.H. Jung<sup>1,2</sup>, P.J. Ainsworth<sup>1,2</sup>*. 1) Cancer Genetics, London Regional Cancer Centre, London,ON,Canada; 2) Cancer Genetics, London Health Sciences Centre, London,ON,Canada.

The discovery of the BRCA genes has provided an increased demand for genetic counselling and testing in families with histories of breast/ovarian cancer. Limited information is available regarding the impact of genetic testing on an individual's life. The purpose of this study was to assess individuals' satisfaction with the genetic counselling and testing process. A questionnaire was sent to 203 individuals from known BRCA1/2 families in Southwestern Ontario asking them to rate the genetic counselling and testing process. All individuals had received their results at least 6 months prior to receiving the questionnaire. Of the respondents, 54.9% were carriers and 45.1% were non-carriers. Eighty-four percent were female and 15% were male with a median age of 48.7 years. Ninety-five percent were extremely satisfied with the genetic counselling they received, 95% would still proceed with testing regardless of current knowledge, and 96% indicated that they would recommend testing to a non-family member. On average the waiting time for test results was 6-9 months (mean 5.8). Sixty-nine percent of respondents were satisfied with this waiting time though 6.9% were extremely dissatisfied. The majority (88.8%) were pleased to receive results in person. Some participants indicated that they would prefer to receive their results by telephone (4.9%), mail (7.8%) and through their family physician (2.9%). Overall, the majority of participants were extremely satisfied with both the genetic counselling and testing process.

**The Psychosocial Impact of Genetic Testing on Members of BRCA1/2 Positive Families.** *G. Sheridan<sup>1</sup>, N. Scanlan<sup>2</sup>, A. Horler<sup>1</sup>, S. Nanda<sup>1</sup>, J.H. Jung<sup>1,2</sup>, P.J. Ainsworth<sup>1,2</sup>.* 1) London Regional Cancer Centre, London, Ontario, Canada; 2) London Health Sciences Centre, London, Ontario, Canada.

With the discovery of the breast cancer susceptibility genes, BRCA 1/2, there is a greater demand for genetic counselling and testing of individuals at risk. Very little information is available regarding the psychosocial impact of BRCA testing on an individual's life. The purpose of this study was to determine how genetic testing has affected the lives of individuals belonging to a BRCA mutation positive family. Psychosocial questionnaires were mailed to 203 individuals in Southwestern Ontario at least 6 months after they received their test results. There were 102 respondents belonging to 44 different BRCA positive families. Fifty-five percent were carriers and 45% were non-carriers. Carriers reported that they were angry (17.9%), anxious (35.7%), worried (50%) and depressed (16.1%) after they received their results. At the time of the questionnaire, carriers reported that they felt worried (41.1%) and relieved (25.0%). Non-carriers reported that they were relieved (84.8%) after they received their results. At the time of the questionnaire, 69.9% reported that they were still relieved. Four percent reported that they experienced feelings of guilt. The majority of participants (88.2%) shared their result with one or more immediate family members. Seventy-two percent also shared their result with one or more members of their extended family. Thirty-seven percent shared their result with a family member under the age of 18. Individuals reported that family members' reactions to the results varied. Carriers reported that their family members experienced feelings of guilt, anger, anxiousness and fear. Non-carriers reported that their family members experienced feelings of relief, non-concern and guilt. Overall the results of this survey appear to be consistent with other studies that have been reported. The survey was based on a small population size and was limited to one geographic location in Ontario. It may be beneficial to have a similar multicentre survey to include the entire province of Ontario.

**Parental coping with Proteus syndrome: A chronic progressive disorder.** *J.T. Turner<sup>1</sup>, B.B. Biesecker<sup>1</sup>, J.R. Leib<sup>2</sup>, L.G. Biesecker<sup>1</sup>, K.F. Peters<sup>3</sup>.* 1) National Human Genome Research Institute/National Institutes of Health, Bethesda, MD; 2) National Institute of Neurological Diseases and Stroke/National Institutes of Health, Bethesda, MD; 3) Department of Biobehavioral Health, Pennsylvania State University, University Park, PA.

Little is known about how parents cope with raising a child with Proteus syndrome, a progressive disfiguring overgrowth condition. The psychosocial consequences of this condition are likely analogous to other progressive genetic conditions. Genetic counseling seeks to enhance clients ability to adjust to their child's condition by assisting clients in identifying and utilizing their coping resources. The purpose of this qualitative study was to learn how parents perceive Proteus syndrome and how health professionals can help to facilitate their coping process. Qualitative interviews with 31 parents, average age 35.6 years, were conducted using a semi-structured guide developed using Goffman and Jones stigma delineations. Codes used for analysis were derived from recurring themes that emerged from the data. Coping strategies depicted in Carver, Weinstraub, and Scheiers COPE inventory tool and answers to targeted questions were assessed. Three independent coders validated the coding scheme and there was high inter-coder reliability. Parents were found to utilize a combination of coping mechanisms including problem and emotional focused strategies. The majority was adaptive and 76% reported to have come to accept their child's condition. The most common coping mechanisms described include seeking emotional support (68%), positive reappraisal and growth (67%), active coping strategies (66%), downward comparison (58%), turning to religion (52%), seeking instrumental support (57%), and restraint coping (48%). Less than 3% reported using maladaptive coping mechanisms such as alcohol or other substance abuse. While 25% described mental disengagement, <10% reported behavioral disengagement. These findings provide insight into the avenues genetic counselors may explore with their clients to facilitate coping and overall adjustment among parents of children with progressive disfiguring conditions like Proteus syndrome.

**Feasibility and utility of the Colored Eco-Genetic Relational Map(CEGRM) in women at risk for hereditary breast cancer.** *J.A. Peters<sup>1</sup>, R. Kenen<sup>2</sup>, R. Giusti<sup>1</sup>, J. Loud<sup>1</sup>, N. Weissman<sup>1</sup>, M.H. Greene<sup>1</sup>.* 1) Div Cancer Epid & Genetics, NCI/NIH/DHHS, Rockville, MD; 2) Sociology Dept.The College of New Jersey, Ewing, NJ.

Genetic counseling involves not only the provision of genetic information, but also helps at-risk individuals integrate new genetic information, adjust to it emotionally and socially, and make informed decisions. To address these latter goals, we conducted a pilot test of the CEGRM (Colored Eco-Genetic Relational Map), a novel, recently-developed psychosocial assessment tool, which combines features of the genetic pedigree, family systems genogram, and ecomap (Kenen & Peters, JGC, 2001). The CEGRM presents a simple, concise, visual representation of the social interaction domains of information, services, and emotion exchanges through the application of color-coded symbols to the genetic pedigree. The process of completing the CEGRM was designed to facilitate contemporary genetic counseling goals of: (a) understanding the client in the context of her/his social milieu; (b) bolstering client self-awareness and insight; (c) fostering active client participation and mutuality in the counseling interaction; (d) eliciting illuminating family history social narratives; and (e) addressing outstanding emotional issues. Twenty participants in a breast imaging study of women from families with BRCA1/2 mutations completed and evaluated various aspects of the CEGRM. We found that the efficient construction of the CEGRM was feasible, and that compliance was excellent. Participants developed insights into themselves and their social milieu through the pattern of relationships revealed by the CEGRM's visual display. The process of co-constructing the CEGRM fostered the participant's active involvement in the genetic counseling session, which was noteworthy for mutuality of interaction between participant and researcher. In this context, the participants felt free to share poignant social narratives about their friends and families. Further studies are planned to refine the CEGRM, and to examine its utility in clinical cancer genetics research.

**Cumulative Clinical and Molecular Diagnostic Experience of 361 Patients in the Genetics of Hearing Loss Clinic.**

*D. Yaeger<sup>1</sup>, K. Lewis<sup>2</sup>, L. Soslow<sup>2</sup>, U. Shah<sup>3</sup>, J. McCallum<sup>1</sup>, L. Sellen<sup>1</sup>, C. Stolle<sup>4</sup>, I. Krantz<sup>1</sup>.* 1) Division of Human Genetics and Molecular Biology; 2) Audiology; 3) Otolaryngology; 4) Clinical Laboratories, The Children's Hospital of Philadelphia, PA.

Hearing loss (HL) occurs in approximately 2 out of every 1000 births and is genetic in origin in approximately 50% of cases. This high incidence, coupled with the recent discovery of genes involved in hearing loss and the trend toward universal newborn screening, has led to the establishment of the Genetics of Hearing Loss Clinic at The Children's Hospital of Philadelphia. We present our experience in this clinic over the past four years. To date 361 individuals have been evaluated, most having been referred by an Otolaryngologist and/or Audiologist. Of these 361 patients, 279 (77.3%) had bilateral HL and 82 (22.7%) had unilateral HL. To determine the cause of their hearing loss and screen for syndromic forms of hearing loss, individuals were offered a panel of tests. Depending on the type and severity of the hearing loss, recommendations included GJB2 (Connexin 26) mutation analysis, renal and thyroid function studies, a CT scan of the temporal bones, an ophthalmology evaluation, and an EKG. Of the 361 patients evaluated 59 (16.3%) had a syndromic etiology for their hearing loss assessed either through physical exam, medical history, or abnormal test results. Forty-two (71.2%) of these had bilateral HL and 17 (28.8%) had unilateral HL. Twenty-five different syndromic etiologies were identified, the most common of which included Waardenburg syndrome, Usher syndrome, Mire's disease, Pendred syndrome, Stickler syndrome, and 18q- syndrome. Genetic testing of the GJB2 gene was completed for 219 (78.5%) patients with bilateral sensorineural HL, 26 (11.8%) of which were found to carry two mutations in the GJB2 genes and an additional 26 (11.8%) were found to be heterozygous for a mutation in the GJB2 gene. Seventeen different variants were identified in the GJB2 gene, the most common being 35delG, M34T, V37I and 167delT. Proper clinical evaluation in the context of a hearing loss clinic combined with molecular testing leads to more appropriate patient care and medical management.

**Development of two decision aids for genetic testing for hereditary cancer.** *C. Wakefield<sup>1, 2</sup>, B. Meiser<sup>2</sup>, J. Homewood<sup>1</sup>, E. Lobb<sup>2</sup>, J. Kirk<sup>3</sup>, K. Tucker<sup>2</sup>.* 1) Department of Psychology, Macquarie University, NSW, Australia; 2) Department of Medical Oncology, Prince of Wales Hospital, Randwick, NSW, Australia; 3) Familial Cancer Service, Department of Medicine, Westmead Hospital, Westmead, NSW, Australia.

Background: The demand for genetic counseling and genetic testing for individuals with a family history of cancer is considerable and increasing. Current best practice in genetic counseling may not allow a full deliberation of the consequences of decisions about genetic testing for cancer susceptibility. Literature on risk perception in high-risk breast cancer families reveals persistent over-estimation of risk, even after counseling. In this study, two decision aids were designed to assist people to participate actively with practitioners in deciding about genetic testing for cancer susceptibility. Methods: Stage 1. We present the development of two decision aids for individuals considering genetic testing for breast/ovarian cancer and hereditary non-polyposis colorectal cancer (HNPCC) susceptibility. The aids were developed by a team of experts guided by expectancy-value decision theories and the literature on risk communication and the psychological impact of genetic testing. Results: Stage 1. Two 20 page full-colour decision aids were developed using varying formats of words, numbers, graphs, pie-charts and illustrations. They address a) the population risk of breast or bowel cancer b) inherited breast cancer or HNPCC c) the cumulative risk for patients with high risk genetic mutations d) the individual's suitability for genetic testing e) the differences between mutation search and predictive testing f) the potential benefits and risks of genetic testing and g) screening and management options. Fold-out charts ask individuals to rate the importance of each potential risk and benefit as a leaning towards or against having the genetic test. Stage 2: (yet to be undertaken) involves two concurrent randomised controlled trials of the decision aids, each with 120 patients, assessed by questionnaire at two time points after genetic counseling. The decision aids and data supporting its development will be presented.

Program Nr: 1113 from 2003 ASHG Annual Meeting

**The Living Genome: Reading the Book of Life.** *R. Alford<sup>1</sup>, C. Sumners<sup>3</sup>, S. Wright<sup>3</sup>, K. Henderson<sup>3</sup>, C. Maxwell<sup>3</sup>, D. Gassmann<sup>3</sup>, K. Ghonima<sup>3</sup>, K. Bradley<sup>3</sup>, H. Valdes<sup>3</sup>, D. Brooks<sup>3</sup>, N. Greig<sup>3</sup>, J. Wise<sup>3</sup>, D. Van Tuerenhout<sup>3</sup>, D. Temple<sup>3</sup>, K. Morris<sup>2</sup>, M. Rives<sup>2</sup>, S. Scherer<sup>2</sup>, G. Weinstock<sup>2</sup>, R. Gibbs<sup>2</sup>, R. McDonald<sup>3</sup>.* 1) Otorhinol & Communicative Sci, Baylor Col Medicine, Houston, TX; 2) Human Genome Sequencing Center, Dept Molecular & Human Genetics, Baylor Col Medicine, Houston, TX; 3) Houston Museum of Natural Science, Houston, TX.

**The Living Genome: Reading the Book of Life** is a 3,000 sq ft genetics/genomics exhibit at the Houston Museum of Natural Science (HMNS). The exhibit is a partnership between the HMNS and the Baylor College of Medicine Human Genome Sequencing Center. The exhibit is divided into nine content areas including the history of genetics/genomics, the structure and function of DNA, inheritance, the Human Genome Project, comparative genomics, genetically modified organisms, medical genetics, biotechnology, and genetics in natural science. The exhibit invites visitors to learn the science of genetics/genomics; explore the ethical, legal and social challenges presented by recent discoveries; and, consider the impact of new knowledge and technologies. The exhibit incorporates six video displays, one theater, four interactive computer kiosks, and six hands-on activities. At the exhibit's exit, 30 questions are posed through three polling stations. Preliminary data predict surprising findings: 44% said the patenting of genes should not be allowed; 45% would be concerned if their employer learned of presymptomatic genetic test results; 46% believe their insurance company should not know if they carry a gene that places them at risk for future disease; 29% stated genetic information is never different from other personal health information; 45% stated that it is acceptable to use gene therapy to treat disease, modify physical traits, increase intelligence, or confer special abilities; 55% stated human cloning would never be acceptable; 66% would not clone a pet; 51% support human stem cell research while 19% said that they would only support such research if stem cells come from adults; and, 42% believe newborn screening programs should report any disease discovered, regardless of whether it is treatable. Data collection will continue through December 2003.

**Multi-Institutional Survey to Assess the Need for Genetic Counseling in Pediatric Cardiology Settings. D.**

*Gruber<sup>1</sup>, R.S. Cooper<sup>2,5</sup>, J.D. Menasha<sup>3</sup>, M. Rutkowski<sup>4</sup>, D.T. Hsu<sup>5</sup>, J.G. Davis<sup>6</sup>, J.G. Pappas<sup>7</sup>.* 1) Biological Sciences, Columbia University, New York, NY; 2) Department of Pediatrics, Division of Pediatric Cardiology, Weill Cornell Medical Center of New York Presbyterian Hospital, New York, NY; 3) Department of Human Genetics and Pediatrics, Mount Sinai School of Medicine, New York, NY; 4) Department of Pediatrics, Division of Pediatric Cardiology, New York University School of Medicine, New York, NY; 5) Department of Pediatrics, Division of Pediatric Cardiology, Columbia Presbyterian Medical Center, New York, NY; 6) Department of Human Genetics, Weill Cornell Medical Center of New York Presbyterian Hospital, New York, NY; 7) Department of Pediatrics, Human Genetic Program, New York University Hospital Center, New York, NY.

The continuous advancements in human genetics have changed the delivery of care in many fields of medicine, including Cardiology. Among the many roles of the Genetic Counselor, gathering pertinent medical information, explaining genetic concepts, and helping patients and families deal with the psychosocial implications of these diseases are of utmost importance. In most institutions, the Cardiologists themselves perform these functions. This study sought to assess the level of training in and the knowledge base of genetics among Pediatric Cardiologists, as well as to assess their attitudes towards genetic testing and genetic counseling. The probability sample of pediatric cardiologists (N=610) was randomly selected from 30 geographically representative states. 81 (14.4%) completed surveys were analyzed. This project also includes an ongoing survey that seeks to evaluate the genetic counseling needs in pediatric cardiology patients and their families. The study revealed a deficiency in knowledge, among the Cardiologists study group, of genetics and genetic counseling and significant differences in their responses to patients psychological concerns. Additionally, Pediatric Cardiologists frequently did not recognize the importance of certain issues considered essential in a genetic counseling setting, such as anxiety, reproductive options, implications of the results on other family members, support networks and test methodology.

**Genetics and Education in the Public Arena - an Ethical Perspective.** *F. Cunningham*<sup>1,2</sup>, *M. Aitken*<sup>1,2</sup>, *S. Metcalfe*<sup>1,2,3</sup>, *R. Dow*<sup>1,2</sup>, *R. Williamson*<sup>1,2</sup>. 1) Education Unit, Gene CRC, Melbourne, Australia; 2) Genetics Education Unit, Murdoch Childrens' Research Institute, Melbourne, Australia; 3) Dept of Paediatrics, The University of Melbourne, Australia.

Completion of the Human Genome Project is a monumental milestone in the search for genes associated with human disease. It has great implications for medicine, and raises ethical issues for our society as a whole. Despite media speculation, this significant achievement is really the first step in a long journey to unravel the genetic nature of being human. Likewise, we are only at the first step in dealing with the impact these discoveries will have in our society in medicine, law and everyday life.

The Gene CRC recognises the importance of involving the public in debates surrounding genetic technologies in medicine. We have developed an education program aimed to promote an awareness that genetics is an integral part of our society, and that informed debate is essential to make decisions about applications of genetic technologies that will be acceptable in the moral framework of an Australian community. Unique sets of resources and activities have been developed which aim to engage the public to learn more about human genetics through ethical issues. These resources target many audiences, including ethics committees, health professionals and primary and secondary schools; and utilise a diverse range of media. These include a website designed for public education, worksheet kits, a CD-ROM and a unique essay competition for secondary students and teachers, and a manual for ethics committees. The tangible products of this program are being adopted by other genetics education providers and researchers, and have succeeded in raising the level of informed debate on human genetics in the public arena.

**Standardized patient methodology is an effective way for medical students to gain experience in the clinical application of genetic concepts.** *A. Greb, A. Trepanier, M. Kavanagh, G.L. Feldman.* Wayne State University, Detroit, MI.

We previously reported that the standardized patient (SP) methodology is a valuable way for medical students to apply genetic concepts clinically. To continue to evaluate their effectiveness, we report the results of two years experience comparing SP methodology with student role-plays. During a small group session that is part of our medical genetics course, students were assigned one of two cases. In one case, SP's were trained to present the clinical scenario which involved a pregnant woman at risk for carrying a dystrophin mutation (DMD case). For the other case, students were given specific information and instructed to play the role of a 43-year-old woman with a family history of spina bifida (NTD case). In both cases, students were given referral information and told to work as a team. They were expected to ascertain the patients concerns, review lab results, collect targeted history information, perform a risk assessment, explain the genetics and natural history of the condition, offer genetic testing and disclose results. As one group worked through their case the other observed. Observing students documented if the genetic principles inherent in the case were correctly applied. All students were asked to complete an evaluation form assessing their perception of the usefulness of SP's compared to student role-playing (n=326). Seventy-three percent of the students stated that SP's enhanced their learning compared to student role-playing; 71% stated that the SP was a beneficial addition to the DMD case; and 61% stated they would recommend using SP's again. Student comfort level when talking with the patient was also assessed. During the first year of our experience, students 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%). During both years, students commented that they took the encounter more seriously when an SP was the patient. In conclusion, we found the learning experience is enhanced when using the SP methodology as a way for medical students to gain experience applying basic genetic principles clinically.

**Genetics Home Reference: a new federal government resource.** *J. Mitchell*<sup>1</sup>, *C. Fomous*<sup>2</sup>, *S. Morrison*<sup>2</sup>, *D. Mucci*<sup>2</sup>, *S. Davenport*<sup>3</sup>, *A. McCray*<sup>2</sup>. 1) University of Missouri, Columbia, MO; 2) National Library of Medicine, Bethesda, MD; 3) Sensory Genetics, Bloomington, MN.

The Human Genome Project generated a great deal of interest in the promise of genomic medicine but, at the same time, left the public (and many healthcare professionals) scrambling to understand how genetics applies to the prevention, diagnosis, and treatment of disorders and diseases. Although the sequencing data produced by the Human Genome Project are freely available, little of this information or its impact is understandable to the general public. To address this problem, the National Library of Medicine developed an online resource called the Genetics Home Reference (GHR), located at <http://ghr.nlm.nih.gov>.

Launched in April 2003, GHR provides a bridge between the clinical questions of the public and the rich technical data emerging from the Human Genome Project. GHRs integrated web-based approach provides brief, consumer-friendly summaries of genetic conditions and related genes. Understanding is enhanced by direct links to glossary definitions and a resource called Help Me Understand Genetics that explains the basics of genetics. Additional links to consumer information on MEDLINEplus, applicable clinical trials, and relevant patient support groups are provided. Each summary also includes links to advanced sources of information such as LocusLink, GeneReviews, OMIM, and PubMed.

The GHR target audience is the motivated lay public with some understanding of biology, but the website also serves as a tool for a wide spectrum of healthcare professionals. The summaries answer preliminary questions about genes and genetic disorders that might be of interest to patients and their family members, students, journalists, and the curious public. For healthcare professionals, the website provides a central source of information suitable for patients and also links to genetic databases for primary data or detailed discussions. Currently, GHR summarizes single-gene disorders, with long-range plans to include some chromosomal and multifactorial disorders and to address additional health implications of the Human Genome Project.

**Teaching genomic medicine to third year medical students: The Einstein experience.** *R. Marion*<sup>1,2</sup>, *C. Campbell*<sup>2</sup>.  
1) Dept Pediatrics, Montefiore Medical Ctr, Bronx, NY; 2) Sarah Lawrence College, Bronxville, NY.

Genomics will play an increasingly important role in medicine in the future. In the clinical years of medical school, however, little emphasis is placed on teaching molecular medicine, largely due to a paucity of trained personnel. To address this, we have developed a curriculum in which genetic counselors play an active role in teaching basic concepts in genomic medicine. The pilot study, a series of 7 weekly seminars that spans the pediatrics clerkship at the Albert Einstein College of Medicine, was taught by the co-authors. Seminar topics included: clinical and molecular cytogenetics, gene expression, single gene mutations, molecular therapy, triplet repeat disorders, and genetic approaches to common disease. Each session was case-based, often relating to a patient on the wards. In each session, the case was discussed in terms of general pediatric themes (clinical course, pathophysiology, and treatments). However, the specific diagnoses served as a basis for the exploration of concepts such as molecular classifications of disease, diagnostic tools, treatments, and ethical issues in genetic medicine. Prior to each session, students were provided with a summary of the case, as well as background reading material. The curriculum was implemented in July 2002. Verbal feedback indicated that: 1) students favored the didactic approach using inpatient cases; 2) integration of the clinical course and molecular basis of diseases made basic concepts and the practical applications of molecular medicine more comprehensible; and 3) participants appreciated the different perspectives that the pediatrician and genetic counselor brought to the discussion. On written evaluation, the series received extremely high marks from the students. We believe that by combining genetic counselors and clinicians in a team-teaching format, this modular, case-based curriculum can be successfully transported and used to teach genomics to medical students at other institutions and in other specialties. The development of this curriculum creates a new niche for genetic counselors as educators at the medical school level.

**Teaching about genetic disorders: A survey of Resident understanding of Inborn Errors of Metabolism.** *P.A. Levy, R.W. Marion.* Pediatrics - Genetics, Children's Hosp Montefiore, Bronx, NY.

As Genomic Medicine becomes more incorporated into the practice of medicine, how effective are we at conveying the necessary information to our trainees? Each year the residents have lectures about Inborn Errors of Metabolism (IEM). Consult services often ask for genetic testing. Residents are called on to understand which test might be appropriate, how it needs to be collected and to some degree, how to interpret the results. As part of their differential, they should also be expected to include IEM as warranted. We decided to survey Pediatric residents at our institution to assess their understanding of IEM. Questions were asked about newborn screening, genetic testing, and their experience and training with this type of genetic patient. Clinical scenarios were presented to assess recognition of IEM. Results: For newborn screening, the residents had a fair knowledge of which disorders are screened for in our state (NY). 60-70% of the residents could correctly identify one of three tests recently added to the NY newborn screen (MCAD, CAH, CF). With questions dealing with procedural issues related to IEM testing, about 50% correctly identified the type of tube needed for plasma amino acids and for ammonia, 50% knew that a random urine specimen was sufficient for urine organic acids, and 60% understood that using a tourniquet would raise lactic acid levels. The residents were unable to identify a patient presenting with MSUD, could not state that MSUD was an disorder of amino acid metabolism, and could not correctly order plasma amino acids to confirm the diagnosis. 50% knew that Galactosemia is associated with jaundice and E. coli sepsis. Only 4% of the residents recognized a typical presentation of Metachromatic Leukodystrophy. Most of the residents surveyed felt that their teaching and experience with patients with IEM had been deficient. Conclusion: More effort must be made to provide access to information about genetic testing to the residents, possibly over the hospitals intranet web site. Efforts must also be made to increase the residents knowledge about genetic disorders such as IEM so these disorders can be included in their differentials.

**Genetic knowledge and attitudes among individuals at risk for Alzheimer's disease.** *T. Moscarillo*<sup>1</sup>, *R. Go*<sup>2</sup>, *H. Holt*<sup>2</sup>, *S. Goldberg*<sup>1</sup>, *A. Chaglassian*<sup>1</sup>, *J. Stoler*<sup>3</sup>, *D. Blacker*<sup>1</sup>. 1) Psychiatry, Mass General Hosp, Charlestown, MA; 2) Epidemiology, U of Alabama, Birmingham, AL; 3) Genetics and Teratology, Mass General Hosp, Boston, MA.

For those at risk for Alzheimer's disease (AD), the growing complexity of genetic information is a particular concern. To examine baseline knowledge of AD genetics and attitudes toward genetic testing among at-risk individuals and to pilot educational materials for future clinical use, we recruited 247 unaffected relatives in the NIMH AD family sample (mean age 61, sd 9, range 40-90; 25% male; 97% white, 3% black; mean education 15 years, sd 4). Subjects were assessed at baseline, immediately after a randomized educational intervention (AD genetic educational video + pamphlet vs. the pamphlet alone vs. a placebo pamphlet), and 3-6 months later. At baseline, self-assessment of AD genetic knowledge was low (e.g., 58% reported little or no understanding), and answers to specific questions bore this out (e.g., 44% overestimated the increase in risk due to family history). Knowledge of general risk factors was somewhat better (e.g., 93% thought age increased risk and 63% thought vitamin E decreased risk). Subjects recognized the names of some AD genes, but few could correctly state their impact on risk or age of onset. As for genetic testing, 70% said they would be interested in a test offering certain results, but only 59% in a test for disease risk. The most commonly cited reasons for interest were the chance to institute preventive measures, the need to attend to personal affairs, and the desire to prepare loved ones for the future; for lack of interest, the most commonly cited reasons were fear of losing insurance and lack of effective preventive treatments. Qualitative review of follow-data on 44% of the subjects showed little gain in confidence, but real gains in knowledge, particularly in the video group, and somewhat decreased interest in genetic testing. Future results will include complete immediate and long-term follow-up data as well. Educational methods like these will be critical to any future treatment strategies entailing genetic testing for patient selection or treatment optimization.

**Interactive system of Clinical Genetic Semiology: a new pedagogic resource for medicine students.** *F. Suarez, J. Prieto, I. Zarante, C. Gutierrez.* Instituto de Genetica Humana, Univ Javeriana, Bogota, Colombia.

Clinical Genetics have begun to be part of clinical practices in most of the Central and South American hospitals showing the importance of implementing these area in medical education. Therefore new pedagogical tools are needed to include the genetic approach of the diseases in students training. We considered that medical semiology is fundamental in the correct clinical approach of the patient, especially semiology used in clinical genetics, but its teaching becomes difficult by the specialized terminology and the variety of signs that in most of the cases are of difficult definition. The importance of a proper use of semiology is proved in a suitable diagnosis that assures a good treatment. In order to satisfy the necessity of a high quality education in clinical genetics semiology, we presented an educational tool that combines an interactive CD-ROM, a Textbook and Web page, designed in the Instituto de Genetica Humana of Pontificia Universidad Javeriana. The interactive CD-ROM allows the user to compare the findings in his patient with photographies of clinical signs classified by anatomical area and characteristics like color, size, forms, appearance or fusion defects. If the user considers that is the same sign, the one see it in the CD-ROM than the one in the patient, he asks for the software in the CD-ROM, to give information about the sign, from its name, concept, genetic and molecular definition, until its relations with specific syndromes. It is important to stand out that the student arrives at the name and concept of the sign comparing images, which defers from the traditional search in dictionaries or atlas in which the user must know beforehand the name of the sign. The textbook follows the same principle in where the index, guide the user through systems and anatomical areas, that finally show the anomalies and by comparison the student identify the sign, therefore reading the text will known the name the sign and its concept. The Web page combines these characteristics with clinical cases, practical clinical exercises and theory of genetic semiology.

Program Nr: 1122 from 2003 ASHG Annual Meeting

**Web-based Module for Parametric Linkage Analysis.** *S. Slifer, W.K. Scott, M.C. Speer.* Center for Human Genetics, Duke University Medical Center, Durham, NC.

Lod score linkage analysis remains a key analytic technique for localization of trait-associated genes. We have developed an on-line interactive module for instruction in parametric (lod score) linkage analysis. Concepts included are genetic distance and recombination, the use of recombination to identify the location of a disease gene, scoring recombinants and non-recombinants, calculating lod scores, and identifying a minimum candidate interval through the use of multipoint linkage analysis, haplotype analysis, and visual inspection of pedigree data. The site can be accessed at: <http://wwwchg.duhs.duke.edu/Duke2/geneticAnalysis/>. We have also developed a companion module on introductory genetics (<http://wwwchg.duhs.duke.edu/Duke/geneticAnalysis>) which covers, among other topics, meiosis and crossing over, both of central importance to understanding linkage analysis. We gratefully acknowledge support from HG000026 for development of these two modules, and from HP10000 and HP19168 for additional support for the development of the introductory genetics module.

**Attitudes towards psychiatric genetic research and its implication.** *M. Rietschel*<sup>1, 2</sup>, *F. Illes*<sup>1</sup>. 1) Central Inst. Mental Health, Univ. Heidelberg, Mannheim, Germany; 2) Dept. Psychiatry, Univ. Bonn, Bonn, Germany.

### **Objective**

The soon-coming availability of genetic testing in psychiatric disorders raises new and complex ethical issues. Psychiatrists, psychologists, gynecologists, and human geneticists will be in the crucial position to transfer knowledge about psychiatric genetics to their mentally ill patients and families. Differences in attitudes between counselors and their patients could cause misunderstandings and problems.

### **Method**

In a population-based study 3077 persons of the general population were interviewed about their knowledge, attitudes and fears towards psychiatric genetics research and its possible application (e.g. predictive testing). Furthermore, 83 psychologists, 118 psychiatrists, 61 gynecologists, 56 human geneticists, and 316 patients with a DSM-IV diagnosis of a major affective disorder (n=196) or schizophrenia (n=120) were included in the study

### **Results and Conclusions**

The majority (80%) is in favor for psychiatric genetic research. Simultaneously however, more than 50% expressed moral doubts. Whereas the consultants showed a relative homogeneity in their attitudes, these differed significantly from those of their patients and the general population. While only 7% of the human geneticists and only a minority of medical consultants (22% of psychiatrists, 17% of psychologists, and 19% of gynecologists) favored predictive psychiatric genetic testing for themselves, 45% of the general populations and 81% of the patients did so. Testing minors on demand of their parents was favored by none (0%) of the human geneticists, less than 5% of psychiatrists, psychologists, and gynecologists, but by 36% of the general population and 42% of patients. To avoid problems in future consultations, experts have to learn about the specific hopes, fears and objections of their patients and the general population.

**Feasibility study to identify methods for enhancing African American (AA) recruitment to an NCI-funded Cancer Genetics Network (CGN).** *S.J. Olsen<sup>1</sup>, C. Hughes Halbert<sup>2</sup>, K.T. Malvern<sup>3</sup>, C. Smith<sup>2</sup>, C. Griffin<sup>4</sup>, Minority Recruitment Working Group, Cancer Genetics Network, [www.epi.grants.cancer.gov/CGN](http://www.epi.grants.cancer.gov/CGN). 1) Sch Nursing, Johns Hopkins Univ, Baltimore, MD; 2) Univ of Pennsylvania, Philadelphia, PA; 3) National Educational Foundation, Zeta Phi Beta Sorority, Inc (NEFZPhiB), Washington, DC; 4) Sch of Medicine, Johns Hopkins Univ, Baltimore, MD.*

Research documents the challenges of recruiting minority populations to participate in research; reduced participation is even more apparent in cancer genetics research. For example, while prevalence of BRCA1/2 mutations is 12-21% among AAs in high risk clinics, <5% of samples in 3 studies of BRCA1/2 genetic test acceptance were from AAs. Barriers to AA participation in cancer genetics research may include concerns about exploitation, greater levels of psychological distress, reduced knowledge about cancer genetics, and limited exposure to information about inherited cancer. Concerns about exploitation may be heightened in research such as the CGN for which there may be no direct benefit. This feasibility study consists of two sub studies each conducted in different CGN centers. The common outcome is to increase AA enrollment into the CGN. The Penn study is a randomized trial in which potential participants are offered CGN enrollment in the standard fashion vs. an invitation to participate plus participation in a risk assessment program. The Hopkins CGN entered into a collaborative agreement with the NEFZPhiB Sorority whose broad mission includes community education about the Human Genome Project. Standard recruitment is a direct mail campaign to members in 4 states. Enhanced recruitment entails a personalized CGN invitation during a cancer genetics education session at 4 state leadership conferences. Recruitment statistics, Jan-June 2003, include: source of contacts [physician referral (58%), Zetas (42%)] and contacts made [total (n=479); completed CGN enrollment (10%), no f/u (5%), declined (3%), ineligible (1%), pending (81%)]. Lessons learned: there are high levels of interest in cancer risk assessment among AA women; recruitment can build on existing genetics and/or health initiatives. Statistics will be updated.

**Informed Consent for a Population Based Genetic Database: A qualitative assessment of understanding.** *K. Ormond, A. Cirino, R. Chisholm, W. Wolf.* Northwestern University, Chicago, IL.

In November 2002, the NUGene project began recruiting subjects for a large, longitudinal genetic database. Concurrently, we solicited participants for interviews to address attitudes about NUGene participation. Interviews were conducted an average of 22 + 9 days after NUGene enrollment, and 107 transcribed interviews were analyzed (44.6% of NUGene enrollment). Subjects were 58% self-recruited, and mostly female (67%), Caucasian (83.5%), Christian (66.7%), married (48.1%), college educated (66.0%) and with household incomes > \$50,000 (66.3%). Several themes emerged from the data, focusing around reasons for participation, risks/benefits of the study, expectations regarding results and preferences for recontact if future studies or results become available. Most enrolled in NUGene in order to help mankind in some manner, to help find disease genes, treatments or cures, and/or to contribute to the overall scientific knowledge. Less common reasons for participation were a personal interest in science or genetics, or in research participation in general. Many (~30%) clearly expressed a hope for personal benefit, and named specific disorders or family members. Only 10% explicitly stated they had no expectation for personal benefit. Confidentiality protections were described as good by most (>50%), and almost half specifically described one or more of the study's privacy protections. While many were able to articulate the general privacy concerns, and a reasonable minority specifically cited concerns with employer (12%) or insurance discrimination (25%), most considered the risks to privacy low (25%) or none (~60%). When asked whether they expected to be contacted with study results, respondents were split between having no expectation of results (42/107), being hopeful/open to results (40/107) and stating clearly that they expected to be contacted with results (13/107); common explanations were if something I need to know or something serious was discovered. Over 75% of study participants felt that if a genetic test became available for their family they would wish to undertake it; few caveats were mentioned. Implications will be discussed.

**Genetic heterogeneity in a tuberous sclerosis family: implications for genetic counseling.** *A.T. Williams<sup>1</sup>, H. Northrup<sup>1</sup>, K.-S. Au<sup>1</sup>, B.B. Roa<sup>2</sup>*. 1) The University of Texas Medical School at Houston, Department of Pediatrics, Division of Medical Genetics, Houston, Texas; 2) Baylor College of Medicine, Baylor DNA Diagnostic Laboratory, Department of Human and Molecular Genetics, Houston, Texas.

Tuberous sclerosis complex (TSC) is an autosomal dominant neurocutaneous disorder characterized by the development of hamartias and hamartomas in multiple organ systems. The incidence of TSC is approximately 1 in 6000. Mutations occur in either the *TSC1* or *TSC2* genes. One-third of cases are due to inherited mutations and two-thirds are due to *de novo* mutations. We describe a family with distinct mutations identified in two affected members. Research based sequence analysis of the proband (Hou50-01) identified a 22 base pair deletion mutation in the *TSC2* gene (3762\_3783del). The mutation was confirmed at the Baylor DNA Diagnostic Laboratory, where subsequent analyses determined that the proband's mildly affected mother was mosaic for the *TSC2* deletion mutation, while the clinically unaffected maternal grandmother and maternal great-grandparents were negative. A maternal great aunt, clinically suspected to have TSC, underwent sequence analysis of the coding regions of both *TSC1* and *TSC2* at Athena Diagnostics, Inc. Her results were negative for the *TSC2* mutation identified in Hou50-01; a nonsense mutation in the *TSC1* gene (1498 C>T, R500X) was discovered. The *TSC1* mutation is not present in Hou50-01; further familial analyses for the *TSC1* mutation are pending. Our case is the second report of genetic heterogeneity influencing the interpretation of a TSC pedigree and potentially complicating the provision of genetic risk assessment and counseling. Given the frequency of *de novo* events in TSC, both locus and allelic heterogeneity should be considered in the evaluation of extended families with TSC. To ensure the provision of accurate genetic counseling, providers should consider utilizing complete sequencing of both TSC genes in lieu of targeted analyses on a case-by-case basis. This approach may be particularly relevant in pedigrees suggesting non-penetrance or when targeted mutation evaluations yield unexpectedly negative results.

**Impact of direct-to-consumer advertising for BRCA1 and BRCA2 testing on genetic services at a Managed Care Organization.** *J. Mouchawar*<sup>1</sup>, *S. Alford-Hensley*<sup>3</sup>, *S. Laurion*<sup>2</sup>, *D. Ritzwoller*<sup>1</sup>, *R. Meenan*<sup>4</sup>, *J. Ellis*<sup>1</sup>, *A. Kulchak-Rahm*<sup>1</sup>, *M. Finucane*<sup>4</sup>. 1) Clinical Research Unit, Kaiser Permanente Colorado, Denver, CO; 2) Boulder, CO; 3) Henry Ford Health System, Detroit, MI; 4) Centers for Health Research, Kaiser Permanente, Portland, OR.

In September, 2002, Myriad Genetic Laboratories, Inc. initiated an intensive five-month direct-to-consumer advertising campaign for genetic testing for the BRCA1 and BRCA2 genes (DTC) using television, radio, and print media, projected to reach more than 90% of metro Denver homes an average of 16.5 times each. This study describes the genetic counseling and testing impact of Myriads marketing campaign within two Managed Care Organizations, Kaiser Permanente Colorado (KPCO) in Denver, Colorado, where the ad campaign occurred, and Henry Ford Health System (HFHS) in Detroit, Michigan, where there were no advertisements. At KPCO, referrals increased 300% (p-value <0.001) during the advertising campaign, compared to the same time period a year earlier, and the proportion of high risk referrals dropped from 70% the previous year to 49% during the campaign (p-value <0.001). There was no significant change in mutation risk status of women who underwent testing between the 2 time periods. HFHS reported no significant change between the two time periods for numbers or risk status of referrals, or for risk status of women tested. Direct-to-consumer advertising of genetic testing for breast cancer risk resulted in a significant increase in counseling referrals, even for women who did not have a profound enough family history to be considered for testing, but no increase in low risk women actually being tested. Future DTC may create a challenge of how to identify high-risk members in the face of a potential large-scale counseling demand of lower-risk members driving costs and delaying access to higher-risk women.

**Oppenheims dystonia: The challenging genetic counseling associated with prenatal diagnosis.** *T.C. Falik-Zaccai<sup>1</sup>, L. Shachak<sup>2</sup>, M. Khayat<sup>1</sup>, M. Frydman<sup>3</sup>, Z. Borochowitz<sup>2</sup>, N. Giladi<sup>4</sup>.* 1) Med genet, Western Galilee Hosp, Nahariya, Israel; 2) Inst Human Genet, Bnai-Zion Med Cent, Haifa; 3) Inst of Human Genet, Shiba Med Cent, Tel Aviv; 4) Movement Disorders Unit, Sourasky Med Cent, Tel Aviv, Israel.

Oppenheims dystonia (OD) is an autosomal dominant (AD) movement disorder with a variable phenotype and penetrance of 30-40%. It is characterized by early onset, sustained, involuntary muscle contractions that cause significant morbidity. The identification of the causative GAG deletion in the DYT1 gene, enabled prenatal diagnosis (PND). We have ascertained 53 OD affected individuals within 28 families, confirmed molecularly. Five couples requested PND in which three men were non-manifesting carriers and two women affected with OD. Comprehensive genetic counseling was provided twice by a medical geneticist, genetic counselor and a movement disorder specialist before prenatal testing and when results were delivered. Seven PNDs were performed. Three fetuses of three couples were found not to carry the mutation. One of those requested not to be informed the result. Four fetuses of two couples were found to carry the GAG deletion. One couple decided to continue the pregnancy although the mother is a manifested carrier. The second couple, in which the father is a non-manifesting carrier, had three fetuses carrying the deletion and two affected children born previously. They decided to terminate the first two pregnancies but to continue the third. The two affected women practiced uncontrolled body movements during the PND procedure and report that the obstetricians were not aware and impatient to their difficulties. Marital problems arose in at least two couples. Genetic counseling in OD is challenging and presents intellectual, emotional, and moral dilemmas. The fact that only one parent is "responsible", that inheriting the mutation does not necessarily mean being affected, and the modern treatments that allow most patients a relatively good quality of life, contribute to its complexity. A multidisciplinary approach and possibly preimplantation diagnosis are suggested to best meet the needs of couples at risk.

**What's the story? Narrative styles of people living with intersex conditions.** *S. Metcalfe*<sup>1,2</sup>, *E. Hughes*<sup>1,2</sup>, *M. Sahhar*<sup>2,3</sup>, *Murdoch Childrens Research Institute Sex Study Group*. 1) Genetics Education Unit, MCRI, Parkville, Australia; 2) Dept Paediatrics, The University of Melbourne, Australia; 3) Genetic Health Services Victoria, Parkville, Australia.

Intersex is a group of biological conditions with various aetiologies, primarily genetic, where the affected person's sex does not easily fit into one of the two accepted categories of male and female. Current management often involves early surgery to 'fix' genital appearance, however, this is a subject of controversy among the intersex community. A study is being carried out to look at psychological outcomes of people with intersex and their attitudes towards issues in current management, to inform best practice. Part of this study involves interviewing participants to tell their story about living with their condition. It has been suggested by Frank\*, a sociologist, that people who experience an illness tell their story in one of several styles. We are investigating whether those illness narrative styles can be applied to people's stories of intersex conditions and whether they can be a useful listening tool for health professionals. Stories were collected through in-depth interviews with 5 people with intersex conditions, followed by written and telephone correspondence. The data were triangulated and enriched by commentary from treating doctors. The narratives were analysed using Frank's theory, enabling the style of each participant's story to be identified. It was found that each participant told a distinct style of story, even when superficially their experiences seemed similar. Thus, Frank's narrative styles can generally be applied here, with some modifications. Certain cultures and institutions focus on a particular narrative type, making it difficult to recognise other styles of the story. Certainly there are unspoken elements to a person's story not included in the narrative. But the way they tell the narrative reflects how they want their audience to perceive them and recognition of this should contribute to an empathic relationship of listening for the health professional.\*Frank, A. W. (1995). *The wounded storyteller: body, illness and ethics*. Chicago, University of Chicago Press.

**22q11 Deletion Syndrome: Exploration of Attitudes towards the Disclosure Process of the Increased Prevalence of Psychiatric Illness.** *N. Martin<sup>1</sup>, C. Cytrynbaum<sup>1</sup>, P. AbdelMalik<sup>2</sup>, R. Weksberg<sup>1</sup>, A. Bassett<sup>2</sup>.* 1) Hospital for Sick Children, Toronto, Canada; 2) Centre for Addiction and Mental Health, Toronto, Canada.

22q11 Deletion Syndrome (22qDS), also known as Velocardiofacial or DiGeorge syndromes, is the most common microdeletion syndrome. Several reports have documented a high prevalence of psychiatric illness, especially schizophrenia, in patients with 22qDS. The aim of our research was to explore parental attitudes and genetic counsellors' current practice and perspectives regarding disclosure of the common clinical manifestations of 22qDS, particularly the risk of developing a psychiatric illness. To do this we conducted semi-structured interviews with 4 parents of offspring with 22qDS and schizophrenia, and used standard qualitative methods to examine responses. To investigate genetic counsellors' current practice and perspectives we delivered a questionnaire via established list serves, 54 of which were completed and returned. The results showed that parents and the majority of counsellors felt that information regarding the increased risk to develop a psychiatric illness in patients with 22qDS is important to disclose. However, questionnaire results showed that psychiatric disorders were the least likely to be discussed in the initial counselling session after a diagnosis was made in infancy (41%) and prenatally (72%) compared to other features of 22qDS like hypocalcemia (93%; 89%) and learning difficulties (91%; 98%). Parents felt information should be given before psychiatric symptoms are likely to arise. Counsellors' responses were fairly evenly divided between disclosure in infancy (38%), childhood (31%) and adolescence (31%). Counsellors also revealed that they found the discussion of psychiatric issues with parents challenging due to the stigma associated with mental illness, and lack of knowledge about psychiatric illness and treatment. Studies show that early diagnosis and treatment for schizophrenia and related disorders are likely to lead to improved outcome. Counsellors should disclose information regarding prevalence and treatability of psychiatric illness in 22qDS prior to onset of symptoms, which may occur as early as childhood.

**Effects of guided imagery on women considering BRCA1/BRCA2 testing: A feasibility study.** *B.A. McGregor<sup>1</sup>, A. Fishbach<sup>1</sup>, D.J. Bowen<sup>1</sup>, D. Valley<sup>1</sup>, E. Ludman<sup>2</sup>, K.A. Leppig<sup>2</sup>, L. McAuliffe<sup>2</sup>.* 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Group Health Cooperative, Seattle, WA.

Women at high familial risk for breast cancer report short and long-term distress about their risk for cancer. This has been shown to interfere with comprehension of risk information and effect adherence to breast cancer screening guidelines. There is evidence from several studies in numerous medical and non-medical settings that guided imagery interventions are beneficial in reducing distress and increasing coping. In a feasibility study, we adapted guided imagery for women at high risk for breast/ovarian cancer in women considering genetic testing for BRCA1/2. Subjects were eight women from the genetic counseling clinic at Group Health Cooperative in the Seattle/Tacoma area. The intervention was introduced following a general genetic education session (which did not include individualized breast, ovarian cancer or BRCA1/2 risk estimates). Women were asked to listen to a guided imagery tape twice a week for the next 8 weeks. The intervention was delivered to all the women, who responded favorably to the intervention experience. We examined the short-term effects and acceptability of a guided imagery intervention among these women. We measured psychological variables at baseline and two months post-intervention. These women report elevated perceived risk for breast and ovarian cancer than expected. They also report elevated risk estimates for breast cancer in women in the general population. These women overestimate their risk to carry a BRCA1/2 mutation, compared with BRCApro risk estimates. They report higher than average cancer worry scores and elevated anxiety. We will also present results related to changes in distress, coping, optimism, social support, health related quality of life, genetics comprehension and knowledge level, intention to pursue genetic counseling and/or genetic testing, satisfaction with testing choice and breast and ovarian cancer screening intentions. These preliminary data provide support for further studies using guided imagery as an adjunct to standard genetic counseling.

**Knowledge of non-disease genetic traits and the effect on self-concept.** *H.A. Gordish-Dressman<sup>1</sup>, E.S. Gordon<sup>1</sup>, J.M. Devaney<sup>1</sup>, L.S. Pescatello<sup>3</sup>, B. Kelsey<sup>3</sup>, G. Gianetti<sup>3</sup>, P.M. Clarkson<sup>2</sup>, M.J. Hubal<sup>2</sup>, P.M. Gordon<sup>4</sup>, E.E. Pistilli<sup>4</sup>, E.P. Hoffman<sup>1</sup>.* 1) Center for Genetic Medicine, Children's Natl. Med. Center, Washington, DC; 2) University of Massachusetts Amherst, Amherst, MA; 3) University of Connecticut, Storrs, CT; 4) West Virginia University, Morgantown, WV.

With the recent media attention given to genetics and the Human Genome Project, more and more people are becoming aware of genes and their possible consequences. As information on non-disease genetic traits emerges, the social and psychological impact of this information on an increasingly aware public must be addressed. Here we present data from 135 healthy volunteers and the effect knowledge of non-disease genetic traits has on their self-concept. The subjects were part of an ongoing four-year study titled Functional SNPs Associated with Muscle Size and Strength (FAMuSS). This subset of subjects was given a series of standardized assessments of self-concept (the Tennessee Self-Concepts Scale and the Health Orientation Scale) at the time of enrollment. Each subject donated a blood sample for genotyping of four SNPs (ACE, CNTF, gamma sarcoglycan, and UCP-2) previously shown to be associated with human muscle size and/or strength. After completion of a 12-week supervised exercise regimen, subjects were given information on which SNPs they possessed and what muscle size/strength traits those SNPs were associated with. Assessments of self-concept were then repeated to determine what effect, if any, the non-disease genetic knowledge had. When changes in self-concept measures were compared between subjects who possessed only negatively/neutrally associated SNPs and subjects who possessed positively associated SNPs, several significant differences emerged. The two groups showed significantly different changes in physical self-concept ( $t=2.98$ ;  $p=0.015$ ) and in total self-concept ( $t=2.33$ ;  $p=0.021$ ). The groups also showed significantly different changes in health esteem and confidence ( $t=2.00$ ;  $p=0.046$ ). These results suggest that non-disease genetic information does have an effect on self-concept, making it an issue that will need further study as an increasing amount of non-disease genetic information becomes available.

**Knowledge of genetics relevant for daily practice among nearly graduated MDs.** *M.J.H. Baars<sup>1</sup>, A.J.J.A. Scherpbier<sup>2</sup>, L.W. Schuwirth<sup>3</sup>, L. Henneman<sup>1</sup>, A.M.J.J. Verweij<sup>4</sup>, M.C. Cornel<sup>1</sup>, L.P. ten Kate<sup>1</sup>.* 1) Department of Clinical and Human Genetics, VU University Medical Center, Amsterdam, The Netherlands; 2) Institute for Medical Education, University Maastricht, The Netherlands; 3) Department of Educational Development and Research, University Maastricht, The Netherlands; 4) Department of Medical Education, VU University Medical Center, Amsterdam, The Netherlands.

**Objective** To investigate the knowledge of genetics relevant for daily practice among nearly graduated medical doctors (MDs) in the Netherlands. **Methods** A genetic computer exam was designed by Clinical Geneticists (CGs) and Educational Specialists consisting of 215 questions divided over 194 case-histories and divided into three categories: a) essential for daily practice, b) desirable and c) specialized. By assigning one point for each correct answer a total exam score was calculated, expressed as the percentage of questions answered correctly. Three sub-scores were created for the above mentioned categories (a-c). After a validation study among 11 Non-Medical students, 11 nearly graduated MDs, and 10 CGs, showing ascending scores as expected from these groups, the exam was completed by a study sample of 291 nearly graduated MDs from 7 Medical Schools. **Results** The study sample of MDs scored 62% (SD 6.9; 95% CI 61-63%) correct on the total exam score. In the essential knowledge category (a), none of the MDs scored 90% or more correctly and only 15% of the MDs scored over 80% correctly. Not more than a quarter answered 60% of the desirable knowledge questions (b) correctly. Half of the students scored less than 44% correctly in the specialized genetic knowledge category (c). **Conclusions** The results suggest that nearly graduated MDs lack genetic knowledge necessary for daily practice. It is desirable that the genetic knowledge of MDs is upgraded in their postgraduate training and future MDs training programmes should focus more on this topic.

**Development of A Self-Assessment and Educational Tool Regarding Hereditary Breast Cancer Incorporating Views of at-Risk Women.** *W. Cohn<sup>1</sup>, S.M. Jones<sup>2</sup>, S. Miesfeldt<sup>3</sup>*. 1) Health Evaluation Sci, Univ Virginia, Charlottesville, VA; 2) Cancer Center, Univ Virginia, Charlottesville, VA; 3) Division of Hematology/Oncology, Department of Internal Medicine, Univ Virginia, Charlottesville, VA.

This 5-year study with 3 components examined the views of early-onset breast cancer (BC) survivors from Virginia regarding hereditary breast cancer (HBC) with the goal of developing self-assessment and educational materials for women and their clinicians. 1) 22 women with suspected HBC from varied racial and geographic backgrounds were interviewed to assess views concerning HBC. Addressed were: cancer treatment experiences; disease causation; risk perception for self/relatives; family issues; cancer prevention. We focused on 5 categories emerging from the interviews: causation; expectations of BC in self; expectations of BC in relatives; risk reduction and prevention; family communications. 2) 273 women with early-onset BC (<50 yrs), recruited from the Virginia Cancer Registry, responded to 2 mailed questionnaires. The 1st assessed whether participants had personal/family histories suggestive of HBC. The 2nd examined: health history and experience regarding BC; cancer early detection behaviors; knowledge of BC risk factors; knowledge of HBC; beliefs about BC causes; HBC information needs and use of such information; perception of childrens concerns about cancer risk; perceived information needs for children. 137 (~half) were deemed at risk for HBC. Survey results showed the following: cancer screening behaviors; knowledge about BC risk factors and HBC; concerns about childrens risk; HBC information needs and sources. 3) An instructional design process was used to develop and evaluate an educational brochure to assist un- and under-insured women to assess and understand their potential risk for hereditary breast and ovarian cancer (HBOC). The instructional goals of the brochure were to enable users to: identify and understand risk factors for HBOC; understand the importance of knowing if one is at risk; determine ones potential risk; know resources for women at risk. A companion brochure was developed to enable clinicians to assist women this assessment. NHGRI R29 HG01554.

**GenEd - Genetic education for non-genetic health professionals across Europe.** *C. Benjamin<sup>1</sup>, I. Nippert<sup>2</sup>, H. Harris<sup>1</sup>, K. Challen<sup>1</sup>, C. Julian-Reynier<sup>3</sup>, J. Schmidtke<sup>2</sup>, L.P. Ten Kate<sup>4</sup>, U. Kristofferson<sup>5</sup>, K. Henriksson<sup>5</sup>, E. Anionwu<sup>6</sup>, Y. Poortman<sup>7</sup>, A. Kent<sup>7</sup>, W. Holtzgreve<sup>8</sup>, C. Delozier<sup>9</sup>, R. Harris<sup>1</sup>.* 1) Manchester Uni,UK; 2) Inst fur Humangenetik, Universities of Muenster and Hannover, Germany; 3) INSERM 379, Marseille, France; 4) Free Uni Medical School, Amsterdam, Netherlands; 5) Uni Hospital, Lund, Sweden; 6) Mary Seacole Centre, London; 7) VSOP and GIG; 8) Universitaets-Frauenklinik, Basel, Switzerland; 9) ESHG.

GenEd is an innovative 3-year collaboration collecting data for a needs based assessment in education in genetics for primary health care providers in 11 countries. Information from medicine and midwifery was gathered by direct contact with professionals and available website documentation in 5 countries. Results show that countries have wide variation in content and duration of postgraduate genetic education and that France and Germany have a nationally agreed undergraduate medical curriculum but with minimal genetic education, mainly confined to basic science courses and often not immediately visible. In Sweden, the Netherlands and the UK to a large extent the content is at the discretion of individual universities. Evidence from UK and France show that genetic professionals are influencing the genetic content of curricula. Some specialties have adopted specific genetic educational requirements but many lacked any. We show that within each country many organisations may have nominal responsibility for setting, assessing and implementing education. The Phase II questionnaires surveying GPs, paediatricians, ob/gyns and midwives will be available at the meeting. They address the health professionals' knowledge, attitudes and skills in regard to using genetic information in their practice. Questions also ask about the amount and format of education needed. The creation has been initiated of a European Coalition of Health Professional Education in Genetics (ECHPEG) to facilitate collaboration between e.g. European Health Professional Organisations and Clinical Geneticists (EUMS), patient groups (EAGS), educationalists (ESHG Educational Affairs Committee), policy makers (The Wellcome Trust/ Department of Health UK Strategy) and international groups (NCHPEG).

Program Nr: 1136 from 2003 ASHG Annual Meeting

**Innovative Approach to Prenatal Education.** *T. Grant<sup>1</sup>, N. Callanan<sup>2</sup>, J. Griffith<sup>1</sup>, J.R. Sorenson<sup>1</sup>.* 1) U. of North Carolina Chapel Hill, NC; 2) U. of North Carolina Greensboro, NC.

Interactive Computer Assisted Instruction (ICAI) is an effective educational tool. We have developed an ICAI prenatal genetic education program providing education on multiple marker screening, advanced maternal age and carrier testing for sickle cell anemia, thalassemia, cystic fibrosis, Tay-Sachs and Canavan disease. The program uses text, audio, graphics, & animation. It operates by using a touch screen & allows women to identify topics they want to discuss with their provider. Education units were developed by: 1) review of professional practice guidelines & pamphlets from private & public health OB clinics in North Carolina; 3) Pilot work with the general population; 4) Cognitive Response Interviews (CRI); 5) Usability Tests (UT); & 6) provider interviews.

75 non-pregnant women answered pregnancy risk profile questions, questions regarding preference for medical information & knowledge of prenatal testing. Women were 18-49 yrs, 53% were White, 43% were African-American, & 83% reported having been pregnant previously. We used CRI to investigate content clarity of the educational units. Five women who were 20 weeks or less & at least 18, viewed two units each. From these interviews we learned: a) women understood the content, b) risk estimates should be stated as fractions & percents and c) more graphics & detailed explanation of some terms were needed. Five women who were 20 weeks or less, & at least 18, did UT. Each woman viewed one unit. From UT we learned that women liked the touch screen & audio features & that the program was easy to use. It was found, however, that women wanted to review instructions after they started certain parts of the program & that more detail on how to select topics that will be printed were needed. Thirteen providers reviewed 2 units each. They reported that our ICAI program is informative, easy to understand & use and they would recommend that ICAIs in general become a part of standard prenatal care. Together these data suggest that an ICAI program is an acceptable educational tool by both patients & providers.

**Microsatellite Instability Test Results: Perspective From Patients.** *S.K. Nigon<sup>1</sup>, N.L. Lindor<sup>1</sup>, R.D. Goldberg<sup>1</sup>, D.J. Bowen<sup>2</sup>, A. Roche<sup>2</sup>, G.L. Petersen<sup>1</sup>, S.N. Thibodeau<sup>1</sup>, L.J. Burgart<sup>1</sup>, B.W. Morlan<sup>1</sup>.* 1) Mayo Clinic, Rochester, MN; 2) FHCRC Seattle, WA.

**Purpose:** To determine which individuals with colorectal cancer were interested in knowing results of their tumor microsatellite instability (MSI) testing and immunohistochemistry (IHC) testing for DNA mismatch repair genes; also reasons patients had for choosing to learn results and the impact of results on self-assessed quality of life. Secondary endpoints looked at the effect of the supplemental educational material on the decision and the quality of life. This study was prompted by concern that the complex and ambiguous nature of MSI/IHC testing results might be problematic to convey and stressful to receive.

**Patients and Methods:** Colorectal cancers from 414 individuals from families of variable level of cancer risks, were assayed from MSI and IHC for three DNA mismatch repair gene products. Individuals were invited by mail to learn of their MSI/IHC test results. In the invitation, they randomly received either brief or extended educational materials about the testing and a pre-test survey.

**Results:** Of the 414 individuals, 307 (74%) chose to learn their results. There was no significant difference in interest in knowing test results according to: gender (men 72% vs women 77%), age (participants under 50 [74%] vs over 50 [74%]), educational level (1 year post high school [70%] vs over 1 year [77%]), or family history of colon cancer (participants with no family history [75%] vs participants with family history [72%]). Whether or not individuals received the brief or extended educational piece did not influence the decision. Self-assessed quality of life was not altered by receiving MSI/IHC results.

**Conclusion:** Individuals with colorectal cancer had a high level of interest in learning their individual MSI/IHC test results, and did not seem deterred by the inherent complexity or ambiguity of this information. Regardless of test outcome, results did not adversely affect quality of life. These conclusions held true for all subsets of patients that could be analyzed.

**Lay understanding of familial risk of common chronic diseases: a systematic review and synthesis of qualitative research.** *F.M. Walter<sup>1</sup>, J.D. Emery<sup>1</sup>, D. Braithwaite<sup>1</sup>, T.M. Marteau<sup>2</sup>*. 1) Dept of Public Health & Primary Care, University of Cambridge, UK; 2) Psychology & Genetics Research Group, King's College London, UK.

**Aims:** To systematically review and synthesise the qualitative literature to explore understanding about familial risk held by people with a family history (FH) of cancer, heart disease and diabetes mellitus. To develop a conceptual framework that explains the processes by which such individuals develop and deal with their personal perception of disease risk. **Methods:** 22 qualitative papers were identified by a comprehensive literature search. Standard proformas were used for the appraisal of papers and extraction of key concepts, giving 11 papers for inclusion. A meta-ethnographic approach was used to translate the studies across each other, synthesise the translation, and express the synthesis. **Findings:** 5 interconnected constructs ('diseases running in my family', experiencing the relative's illness, personal mental models of disease, personalising risk and control of familial risk) were synthesised to create a conceptual framework of 3rd order constructs centering on developing a personal sense of vulnerability to a disease. This dynamic process is influenced by the salience of the FH, such as numbers of affected relatives, personal experiences of disease, premature death, perceived patterns of illness relating to gender or age at death, and comparisons between themselves and the affected relative. The developing vulnerability is interpreted using personal mental models of disease causation, inheritance and fatalism. People's sense of vulnerability affects how they cope with, and attempt to control, their perceived familial risk. **Clinical Implications:** Individuals with a FH of a common chronic disease develop a personal sense of vulnerability, and features of the FH which give meaning to familial risk may be perceived differently by patients and professionals. Key areas are identified for health professionals to explore with patients that may influence the effectiveness of both risk communication and disease management strategies. A risk communication consultation model is being developed.

Program Nr: 1139 from 2003 ASHG Annual Meeting

**Contribution of X inactivation studies to genetic counseling in X-linked mental retardation.** *M.L. Jacquemont, V. Raclin, D. Genevieve, S. Lyonnet, V. Cormier-Daire, JP. Bonnefont, A. Munnich.* Departement de genetique, Hopital Necker-Enfants Malades, Paris, France.

X-linked mental retardation (XLMR) is frequently considered in mentally retarded males. Because accurate diagnosis is particularly difficult, studying chromosome X inactivation status in mothers is occasionally helpful. We have reviewed the X-inactivation status of thirty-five mothers of mentally retarded boys. Eight cases were sporadic and twenty-seven were familial. We used a PCR-digestion test at the Androgen Receptor (AR) locus and we compared it to the non digested PCR product to determine the status of the mothers. The significant threshold for non-random inactivation was set to 80 %. Twenty-five women (71 %) had random X inactivation (five familial cases), one (3 %) was non-informative at the AR locus and nine (26 %) had a skewed X inactivation (nine familial cases). However, we observed a discrepant chromosome X inactivation in obligate carriers. Indeed, family history suggested that three mothers were obligate carriers but their X inactivation studies revealed that one of these mothers had skewed inactivation while in the others inactivation was random. These results suggest that genetic counseling based on X inactivation studies should be particularly cautious in XLMR.

**Population-based assessment of risk of other cancers in relatives of prostate cancer cases.** *L. Cannon-Albright.*  
Prof, Medical Informatics, Univ Utah Sch Medicine, Salt Lake City, UT.

We performed a relative risk assessment for cancer of other sites in the first-degree relatives of prostate cancer cases. We analyzed the Utah Population Database (UPDB), a genealogy of Utah pioneers and their descendants record linked to a Utah Cancer Registry (1973-2003). We present estimates of relative risks for 34 cancer sites in relatives of prostate cancer cases. The observed cancers occurring in the relatives of 15,535 prostate cases were compared to internal, cohort and site-specific rates of cancer from the UPDB. 14,096 cases of cancer of all sites were observed in the 135, 947 first-degree relatives, with 9,178 expected; OR = 1.54. Over a 2-fold risk of prostate cancer was observed in first-degree relatives (OR 2.18, CI[ 2.10,2.27]. Those cancer sites with the highest significant OR were testicular (1.71) and brain (1.64). Other cancer sites in significant excess with OR > 1.50 included Hodgkins lymphoma, and cancers of the liver, gallbladder, and thyroid. When only relatives of the 1293 early prostate cancer cases (< 61 years) were considered, over a 3-fold risk of prostate cancer was observed (OR 3.24). Relatives of these early prostate cancer cases were at significant increased risk of stomach cancer (1.77), Non-Hodgkin's Lymphoma (1.47), Leukemia (1.63), kidney cancer (1.80), colon cancer (1.38), and breast cancer (1.27). The 3672 first degree relatives of the 385 "familial" early prostate cases (> 1 1st degree relative affected) were at significant increased risk for rectal cancer (2.57) and soft tissue/connective tissue cancers (3.65). Relatives of the early "non-familial" prostate cases were at significantly increased risk for breast cancer (1.52), colon cancer (1.84), and pancreatic cancer (2.12). A survey of the Utah population suggests that first-degree relatives of prostate cancer cases are at increased risk of developing cancer of other sites. Different patterns are observed when familial prostate cancer cases are considered. Analysis of this unique data resource suggests that consideration of other cancer predisposition genes may be warranted, especially for familial prostate cancer cases.

**Familial Myopia Study.** *G.P. Ibay<sup>1</sup>, B. Doan<sup>1</sup>, L. Reider<sup>2</sup>, D. Dana<sup>2</sup>, M. Schlifka<sup>2</sup>, H. Hu<sup>2</sup>, T. Holmes<sup>2</sup>, J. O'Neill<sup>1</sup>, J.E. Bailey-Wilson<sup>1</sup>, D. Stambolian<sup>2</sup>.* 1) Interited Disease Research Br, NHGRI/NIH, Baltimore, MD; 2) University of Pennsylvania.

**Purpose.** To identify regions of the human genome containing genes responsible for nonsyndromic myopia using pedigrees from four ethnic groups of the Myopia Family Study-- Ashkenazi Jewish, Amish, African- and Chinese-American families.

**Methods.** Cycloplegic and manifest refraction were performed on 39 Jewish and 52 Amish families. Individuals with  $-1.00$  D in each meridian of both eyes were classified as myopic. Genomic DNA was genotyped by PCR with 12 markers on chromosome 12q21-23 and 18p11.3. Parametric and nonparametric linkage analyses were conducted to determine whether these loci are important in families with less severe, clinical forms of myopia. A combined nonparametric analysis was performed by calculating the sum of NPL scores for each family divided by the square root of the total number of analyzed families ( $N=78$ ) across both populations.

**Results.** The overall results of these preliminary studies do not indicate any strong evidence of linkage of myopia in these families to the candidate regions on chromosomes 12 or 18. One Amish family showed nominal evidence of linkage ( $LOD>1.0$ ) to the region previously reported on chromosome 12q; another 3 Amish families each gave  $LOD >1.0$  to 2 markers on chromosome 18p; and 3 Jewish families each gave  $LOD >1.0$  to 5 markers on chromosome 12q. Although these families show marginal evidence of linkage to one of these regions, the results are not particularly strong and could be due to chance. A genome wide scan (GWS) is required to determine whether these families are most likely linked to this region, or whether the mild evidence of linkage observed is just a chance result. A GWS will also allow us to determine if other loci may play a role in myopia susceptibility in these families. Such a GWS is currently underway. Data collection is underway in both African-American and Chinese-American families.

**Evidence for a Free Fatty Acid-related Diabetes Susceptibility Locus on Chromosome 22 in Finnish Subjects.**

*A.U. Jackson<sup>1</sup>, R.W. Watanabe<sup>2</sup>, L.J. Scott<sup>1</sup>, K.L. Mohlke<sup>3</sup>, K. Silander<sup>3</sup>, T.T. Valle<sup>4</sup>, K. Doheny<sup>5</sup>, E. Pugh<sup>5</sup>, J. Tuomilehto<sup>4</sup>, R.N. Bergman<sup>2</sup>, F.S. Collins<sup>3</sup>, M. Boehnke<sup>1</sup>.* 1) Univ Michigan, Ann Arbor, MI; 2) University of Southern California, Los Angeles, CA; 3) National Human Genome Research Institute, Bethesda, MD; 4) National Public Health Institute, Helsinki, Finland; 5) Center for Inherited Disease Research, Baltimore, MD.

Dyslipidemia is a hallmark of metabolic syndrome and a common characteristic of type 2 diabetes mellitus (T2DM). Free-fatty acids (FFA) are thought to be a key component in the regulation of hepatic glucose output. As such, understanding the genes underlying the regulation of FFAs and other lipids may provide unique insights into the genetic predisposition for T2DM and related complex diseases. In the Finland-United States Investigation of NIDDM Genetics (FUSION) study, we analyzed fasting and 2-hour FFA levels from the oral glucose tolerance test and fasting lipids in the 491 non-diabetic offspring (age: 34.97.3, BMI: 25.94.3 (meanSD)) of a cohort of 201 families ascertained for T2DM affected sibling pairs. Mean fasting glucose and insulin were 5.00.5 mM and 65.732.8 pM, respectively. Mean fasting and 2-hr FFA levels were 0.45.14 mM and 0.17.05 mM. We estimated trait heritabilities ( $h^2$ ) and performed quantitative trait locus (QTL) linkage analysis by variance components.  $h^2$  for fasting FFA and 2-hr FFA were 0.13 and 0.30, respectively.  $h^2$  estimates for total, HDL, and LDL cholesterol, and triglycerides were 0.64, 0.34, 0.57, and 0.39, respectively.  $h^2$  estimates were not significantly altered when traits were adjusted for age, sex, and BMI. QTL linkage analysis for 2-hr FFA, adjusted for age, sex, and BMI, in a genome scan based on 392 microsatellite markers, yielded a LOD score of 1.94 on chromosome 22 near marker D22S428. The 2-hr FFA linkage signal overlaps with a previously identified region of strong FUSION T2DM association at and around D22S423 ( $p=.00002$ ) at 0.6 cM from D22S428. We currently are measuring 2-hr FFA levels in additional family members in an effort to identify the variant(s) responsible for T2DM risk and FFA variability in this region.

**A Utah Pseudo-Isolate: Proof of Concept using Longevity.** *N.J. Camp, J.M. Farnham, A. Thomas, L.A. Cannon-Albright.* Genetic Epidemiology, University of Utah School of Medicine, UT.

Utah was founded by Mormon pioneers in the mid 1800s. A Utah Population Database (UPDB) was developed based on genealogical records from descendants of these pioneers. Today the database contains genealogy data on ~2.8 million individuals linked to the Utah Cancer registry and Utah death certificates dating to 1904. For studies of complex disease a genetically homogeneous population is desirable. We investigated the potential of a Utah pseudo-isolate from which high-risk pedigrees for common traits could be ascertained for gene mapping. To create a Utah pseudo-isolate, we selected a set of founders and included all individuals descended only from these founders. To ensure enough chromosomes to allow for complex disease we chose a reasonably large number of founders (~38,000), which reduced the chromosomal diversity to ~12% of that for the full UPDB. This Utah pseudo-isolate contains approximately 280,000 individuals. To investigate whether this resource was useful for pedigree ascertainment, we investigated evidence for familiarity, clustering, and high-risk pedigrees in the pseudo-isolate for the example trait of longevity. We considered an age threshold of >100 yrs, as confirmed on a death certificate, to define longevity, and found 155 such individuals (compared to 359 in the full UPDB). Extremely significant excess familiarity was observed. Estimates of measures of familiarity were more extreme in the isolate than in the full UPDB. In the pseudo-isolate first degree relative risks of 5.9 (95% CI: 3.4, 9.6) and 3.2 (95% CI: 1.6, 5.7) were found for 1st and 2nd degree relatives, and 8.4 (95% CI: 3.3, 17.8) for parent-offspring. We identified 20 pedigrees containing at least 2 individuals older than 100 years. A definition of >95 yrs also yielded significant familiarity and 599 pedigrees. We chose a pedigree with 13 individuals >95 yrs and computed the expected number of individuals >95 yrs (2.65), which indicated a significant excess of longevity in the pedigree. Our preliminary findings confirm that a Utah pseudo-isolate has potential for ascertaining pedigrees with reduced genetic heterogeneity and excess risk to map genes involved in complex t.

Program Nr: 1144 from 2003 ASHG Annual Meeting

**Haploblocks, a software tool for visualization and analysis of haplotype blocks in the human genome.** *M. Zucchelli, J. Kere.* Department of Biosciences, Karolinska Institute, Huddinge, Sweden.

HaploBlocks is a software package for the visualization and the analysis of haplotype block structure in the human genome. Supported by a user friendly graphical interface, HaploBlocks computes different measures of linkage disequilibrium and visualizes them into 1D and 2D plots to graphically display the underlying block structure. A set of features has been developed to apply thresholds over allelic frequencies, smooth or shade linkage disequilibrium maps, zoom into particular regions and dynamically remove/insert markers. A dynamic programming algorithm is built in to decompose the DNA data into a set of continuous blocks and to extract the tagging SNPs and the haplotype patterns. In case/control studies Haploblocks computes both allelic and haplotype disease association in terms of odds ratios and their associated p-values by Fisher exact test. As an extra feature, Haploblocks can compare block structure as well as allelic and haplotype frequencies in different populations. Haploblocks accepts as input data both haplotypes and genotypes in standard linkage format. When genotypes are used, haplotype phase is reconstructed by using SnpHap. Haploblocks is programmed in C++ for platform compatibility and direct connection to NCBI database is under development. Applications to case/control studies with asthma and psoriasis data are shown as examples of the use of the tool.

Program Nr: 1145 from 2003 ASHG Annual Meeting

**Candidate Pathway Approach to Genetic Studies of Complex Traits.** *X. Zhou, JY. Zhao, FC. Arnett, MM. Xiong.*  
Inner Medicine, University of Texas, TX.

Traditional methods for genetic studies of complex traits is to investigate one or few genes at a time. However, Complex biology systems are not just the sum of their individual parts. The function of complex biological systems is carried out not only by genes, but also through various networks such as metabolic networks, genetic networks and protein networks, and their different level of organization. A comprehensive understanding of complex traits requires a novel conceptual framework for describing genetics of complex traits. As alternative method to candidate gene approach to mapping complex trait loci, candidate pathway approach to studying complex traits will be proposed. The test statistic for candidate pathway approach will be developed and the power for detecting candidate pathway which influencing complex traits will be investigated. The proposed methods will be applied to systemic sclerosis disease in native Americans.

**Widespread Purifying Selection at Polymorphic Sites in Human Protein-Coding Loci.** *M. Yeager*<sup>1</sup>, *B. Packer*<sup>1</sup>, *R. Welch*<sup>1</sup>, *A.W. Bergen*<sup>1</sup>, *S.J. Chanock*<sup>1,2</sup>, *A.L. Hughes*<sup>3</sup>. 1) Core Genotyping Facility, NCI/NIH, Gaithersburg, MD; 2) Section on Genomic Variation, Pediatric Oncology Branch, NCI/NIH, Bethesda, MD; 3) Department of Biological Sciences, University of South Carolina, Columbia SC.

Estimation of gene diversity (heterozygosity) can be informative in the investigation of molecular evolution. Analysis of 1442 single nucleotide polymorphism (SNP) loci mapped to 234 genes in an ethnically diverse sample of humans (N=102) revealed consistently reduced gene diversities at SNPs causing amino acid changes, particularly those causing amino acid changes predicted to be disruptive to protein structure. The statistically significant reduction of gene diversity at these SNPs, in comparison to SNPs in the same genes not affecting protein structure, is evidence that negative natural selection (purifying selection) has reduced the population frequencies of deleterious SNP alleles. This in turn suggests that slightly deleterious mutations are widespread in the human population and that estimation of gene diversity even in a sample of modest size can help guide the search for disease-associated genes.

**Associations of osseous dysplasias in Neurofibromatosis 1 (NF1).** *S.A. Alwan<sup>1</sup>, L. Armstrong<sup>2</sup>, P.H. Birch<sup>1</sup>, J.M. Friedman<sup>1</sup>.* 1) Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Eastern Ontario Genetics Program, The Childrens Hospital of Eastern Ontario, Ottawa, CANADA.

The most common sites of NF1-associated osseous dysplasia are the long bones (usually the tibia), vertebrae and sphenoid wing. Such lesions may cause profound clinical consequences, and available treatments are often unsatisfactory. The pathogenesis of osseous dysplasia and its relationship to short stature, a common feature in NF1 patients, are unknown. We examined associations between the occurrence of osseous lesions in 595 NF1 probands from the National NF Foundation International Database using logistic regression. After adjusting for age and gender, we found significant associations between the presence of long bone dysplasia and of dysplastic vertebrae (odds ratio [OR] = 1.9, 95% confidence interval [CI] = 1.0-3.6,  $p = 0.04$ ), the presence of long bone dysplasia and of sphenoid wing dysplasia (OR = 3.0, 95% CI = 1.5-6.2,  $p = 0.003$ ), and the presence of vertebral dysplasia and of sphenoid wing dysplasia (OR = 16.5, 95% CI = 5.6-48.6,  $p < 0.0001$ ). No significant association was found between the height of NF1 patients and the presence of either vertebral or long bone dysplasia. We conclude that some NF1 patients are more likely to develop osseous dysplasia than others and speculate that long bone dysplasia, vertebral dysplasia and sphenoid wing dysplasia in NF1 patients share a pathogenetic mechanism that differs from that responsible for NF1-associated short stature.

**Bone-related genetic markers and risk of osteoporotic hip fracture in Utah men and women.** *M. Willing<sup>1</sup>, R. Munger<sup>2</sup>, T. Beaty<sup>3</sup>, H. Wengreen<sup>2</sup>, N. West<sup>2</sup>, R. Cutler<sup>2</sup>, C. Corcoran<sup>2</sup>.* 1) Dept Ped, Div Medical Genetics, Univ Iowa, Iowa City, IA; 2) Dept Nutrition and Food Sciences, Utah State Univ, Logan, UT; 3) Dept Epidemiology, Johns Hopkins Univ, Baltimore, MD.

We examined associations between bone-related markers in candidate genes and risk of osteoporotic hip fracture in a population-based case-control study in Utah. Patients with hip fracture (cases) were ascertained via surveillance of Utah hospitals and were age-matched to controls. Genotypes for polymorphisms in the vitamin D receptor (VDR), estrogen receptor (ER), androgen receptor (AR), type I collagen genes (COL1A1 and COL1A2), osteonectin, osteopontin, and osteocalcin genes were obtained for 325 male and 789 female cases plus 443 male and 852 female controls. The VDR BsmI bb genotype was associated with a reduced risk of hip fracture in women (odds ratio (OR) = 0.77; 95 percent confidence interval (CI) = 0.63-0.94) but not in men. Three polymorphisms (PvuII, Xba I, and a TA repeat at the 5' end of the gene) in the ER gene were each significantly associated with fracture risk in women, but not in men. The polymorphic (AGC)<sub>n</sub> site in the AR gene showed no association with hip fracture risk in men or women. The SP1 polymorphism for COL1A1 (-/-) was associated with an increased risk of hip fracture in both men and women (OR = 1.61, 95% CI = 1.12-2.32); the COL1A1 RsaI, COL1A2 RsaI, and COL1A2 intron 12 VNTR polymorphisms showed no association with hip fracture risk in either men or women. The osteocalcin C/T promoter polymorphism (-/-) was associated with an increased risk in women (OR = 1.28, 95% CI = 1.05-1.57) but not in men. No associations were observed in either men or women for the tightly-linked osteocalcin marker, D1S3737, or the intragenic CA repeats in the osteopontin and the osteonectin genes. These findings provide evidence that polymorphisms in the VDR, ER, and osteocalcin genes are associated with risk of hip fracture in women and that the variation in the COL1A1 Sp1 gene is associated with greater risk of hip fracture in both men and women.

**Gender-mix and genetics: do they modify birth weight - IQ association?** *C. Derom<sup>1</sup>, E. Thiery<sup>2</sup>, R. Derom<sup>2</sup>, S. Van Gestel<sup>1</sup>, N. Jacobs<sup>1</sup>, R. Vlietinck<sup>1</sup>, J.P. Fryns<sup>1</sup>*. 1) Center for Human Genetics, Katholieke Universiteit Leuven, Leuven, Belgium; 2) Association for Scientific Research in Multiple Births, Belgium.

It is widely known that there is an association between birth weight and childhood IQ. The impact of genetic factors on this association can be determined through the investigation of birth weight and IQ in twins. Differences within dizygotic (DZ) pairs are a function of both genetic and non-genetic factors; differences within monozygotic (MZ) twin pairs are almost completely caused by non-genetic factors.

With the Wechsler Intelligence Scale for Children-Revised (WISC-R), IQ was measured in 596 twin pairs with different intra-pair birth weight. The twins (173 DZ same sex, 169 unlike sex, 254 MZ, aged 8-14 yrs) were selected at random from the East Flanders Prospective Twins Survey. Birth weight was obtained from the obstetrical record.

Comparison in MZ and same-sexed DZ twin pairs between co-twins with lowest and highest birth weight showed no significant association between birth weight and IQ. In male DZ pairs the association was suggestive but not significant. In contrast, the same comparison in opposite-sexed twin pairs resulted in a significant association ( $p=0.02$ ). The effect is more pronounced when the female co-twin is heavier than her twin brother: her mean IQ (105,1) is significantly higher than that of female co-twins with lowest birth weight (IQ 99,6) and significantly higher than the mean IQ of same-sexed female DZ co-twins, whether of highest (IQ 99.2) or lowest (IQ 100.1) birth weight.

These results are very suggestive for a gender-mix effect mediating the association between birth weight and IQ. As co-twins are the same age and share influences such as prenatal factors, parental age and smoking, socio-economic status, parity and gestational age, the influence of these possible confounders is negligible.

**Association of the DRD2 141 Indel Polymorphism with Anorexia Nervosa.** *A.W. Bergen<sup>1,2</sup>, M. Yeager<sup>1,2</sup>, R. Welch<sup>1,2</sup>, K. Haque<sup>1,2</sup>, J.K. Ganjei<sup>1</sup>, C. Mazzanti<sup>3,4</sup>, I. Nardi<sup>3</sup>, M.B.M. van den Bree<sup>1,5</sup>, M. Fichter<sup>6</sup>, K. Halmi<sup>6</sup>, A. Kaplan<sup>6</sup>, M. Strober<sup>6</sup>, J. Treasure<sup>6</sup>, B. Woodside<sup>6</sup>, C.M. Bulik<sup>6</sup>, S.-A. Bacanu<sup>7</sup>, B. Devlin<sup>7</sup>, W. Berrettini<sup>6,8</sup>, D. Goldman<sup>9</sup>, W.H. Kaye<sup>6,7</sup>.* 1) Biognosis U.S., Inc., Gaithersburg, MD (Dissolved); 2) Currently at: Core Genotyping Facility, NCI, Gaithersburg, MD; 3) University of Pisa, Pisa, Italy; 4) Currently at: Surgery Branch, CCR, NCI, Gaithersburg, MD; 5) Currently at: Division of Psychological Medicine, University of Wales, Cardiff, UK; 6) Price Foundation Collaborative Group ([www.anbn.org](http://www.anbn.org)); 7) Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA; 8) Center for Neurobiology and Behavior, University of Pennsylvania, Philadelphia, PA; 9) Laboratory of Neurogenetics, NIAAA, Rockville, MD.

To investigate whether the dopaminergic system plays a role in the etiology of anorexia nervosa (AN) via the dopamine D2 receptor, we investigated association and transmission disequilibrium at seven single nucleotide polymorphisms (SNPs) spanning about 75kb of the gene DRD2. We studied 183 probands with a DSM-IV diagnosis of AN, 457 parents and affected relatives with a DSM-IV eating disorder diagnosis, and 92 unrelated, female, normal weight controls. The -141 C/- insertion/deletion (-141 Indel) polymorphism, previously shown to affect DRD2 transcription efficiency, exhibited statistically significant association with diagnosis in a wide array of analyses: both genotype and allele distributions differed significantly between AN probands and normal weight control individuals; the insertion C allele, which appears to increase expression of D2, is transmitted from parents to their affected offspring at rates significantly greater than that expected by chance; and haplotypes containing the insertion C allele and other SNP variants show even greater transmission distortion. Linkage disequilibrium between the -141 Indel and two exon 7 SNPs was observed over a distance of >50kbp in the AN probands but not in the controls. Genetically transmitted variation in D2 dopamine receptor expression appears to play a role in vulnerability to AN.

This abstract does not represent the opinion of the NIH or the Federal Government.

**Variability in white matter hyperintensity volume in men associated with a polymorphism in the NOTCH3 gene: The Framingham Heart Study.** *A.L. DeStefano*<sup>1,2</sup>, *P.A. Wolf*<sup>2</sup>, *S. Karamohamed*<sup>2</sup>, *A. Beiser*<sup>1</sup>, *L.A. Atwood*<sup>1,2</sup>, *J.M. Massaro*<sup>3</sup>, *R.B. D'Agostino*<sup>3</sup>, *C. DeCarli*<sup>4</sup>, *A. Herbert*<sup>2</sup>. 1) Dept Biostatistics, Boston Univ Sch Public Health, Boston, MA; 2) Dept Neurology, Boston Univ Sch Public Health, Boston, MA; 3) Dept Mathematics and Statistics, Boston Univ, Boston, MA; 4) Dept Neurology and Center for Neuroscience, Univ California at Davis, Sacramento, CA.

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary syndrome characterized by stroke, dementia and diffuse white matter hyperintensity (WMH) present on brain magnetic resonance imaging scans. Mutations in the NOTCH3 gene, a membrane bound ligand in the WNT signaling pathway cause CADASIL. Most CADASIL mutations map to the extracellular domain of the protein. Other NOTCH3 polymorphisms may exist and their role cerebrovascular disease is unknown. We tested 10 single nucleotide polymorphisms (SNPs) in the NOTCH3 gene for association with WMH volume relative to total cranial volume in 952 unrelated stroke-free, dementia-free individuals from the NHLBI Framingham Heart Study. Multiple linear regression was used to evaluate genotypic effects, after adjusting for covariates, including age at MRI, age at MRI squared, and sex. In the total sample including both men and women no association was detected between any of the SNPs considered and WMH relative to total cranial volume, after adjustment for multiple comparisons. Analysis of men revealed significant association with SNP rs1548555 (nominal  $p = .01$ ; adjusted for multiple comparisons  $p = .043$ ). Assuming a dominant model, men with at least one A allele had a significantly higher percent WMH relative to total cranial volume (.0530 %) compared to men with the GG genotype (.0425 %) ( $p = .025$ ). No significant associations were detected in women. SNP rs1548555 in the NOTCH3 gene or a polymorphism in disequilibrium with this SNP is associated with increased WMH in men. Additional variants in this gene are being explored.

**Charcot Marie Tooth (CMT) disease: A founder effect of the R298G mutation in LMNA gene in Algeria and Morocco.** *G. Durosier<sup>1</sup>, H. Azzedine<sup>1</sup>, M. Tazir<sup>2</sup>, N. Birouk<sup>3</sup>, E. Genin<sup>4</sup>, D. Ente<sup>1</sup>, A. Bouhouche<sup>3</sup>, G. Stevanin<sup>1</sup>, D. Grid<sup>5</sup>, A. Brice<sup>1</sup>, E. Le guern<sup>1</sup>.* 1) INSERM U289-NEB, Neurogenetics, Hopital de la Salpetriere, Paris, France; 2) Service de Neurologie, Hopital Mustapha, Algiers, Algeria; 3) Service de Neurologie, Hopital des specialites, Rabat, Morocco; 4) INSERM U535, Hopital du Kremlin Bicetre, Paris, France; 5) BTR-AFM-Genethon, Paris, France.

Charcot Marie Tooth (CMT) disease is a pathological and genetic heterogeneous group of hereditary motor and sensory neuropathies. Two major types have been distinguished: demyelinating and axonal CMT. Only 3 loci account for axonal Autosomal Recessive (AR) CMT on chromosomes 1q21 (CMT2B1/1A), 8q13 (CMT4A) and 19q13 (CMT2B). Recently, the R298G mutation in the LMNA gene was reported on patients with CMT2B1 disease. We selected 60 consanguineous families with axonal ARCMT and perform a GeneScan on 15 microsatellites markers covering the 1q21 locus. Sequencing of the LMNA gene was performed in all the families with putative linkage to 1q21. In these families, the R298G mutation was predominant (74 %), highly suggesting a founder effect in North Africa. Indeed, a common haplotype with flanking markers segregated in these families. The R298G mutation found in the LMNA, is probably the result of a founder effect in North Africans. A computational analysis using the appropriate software's will be used to date the mutation. In the other hand, given the high frequency of R298G mutation in this population, it is therefore worth searching this mutation in families with positive linkage to the corresponding region. The Finalized results will be presented durind the meeting.

**Parkin mutations found in Louisiana Parkinson disease patients but not in those of Acadian ancestry. G.**

*Gaikwad<sup>1</sup>, J. Rao<sup>2</sup>, B.J. Keats<sup>1</sup>*. 1) Department of Genetics, Louisiana State University, New Orleans, LA; 2) Department of Neurology, Louisiana State University, New Orleans, LA.

Parkinson disease is a neurodegenerative disorder in which the motor function deficits are due to lesions in the basal ganglia, especially the substantia nigra. Both environmental and genetic factors contribute to the phenotype. In particular, various deletions and point mutations in the parkin gene on chromosome 6q have been reported in patients with onset of Parkinson disease before 50 years of age. Through the Parkinson disease clinic at LSUHSC we have ascertained 22 patients of Acadian ancestry and 87 non-Acadians. Of the 22 Acadian patients, 9 have affected relatives. The goal of this study is to identify susceptibility loci in Acadian families. We have sequenced exons 1-12 of the parkin gene to identify any deletions or point mutations that may be present in the 109 patients. No deletions or point mutations were detected in the 22 Acadian patients. These results indicate that mutations in the parkin gene are not associated with Parkinson disease in the Acadian population, and we are undertaking a genome screen to localize susceptibility genes in this population. However, point mutations were found in two of the non-Acadian patients. A novel missense mutation (Pro37Leu) in exon 2 was found in one patient. This mutation has not been found in any controls and we are checking affected and unaffected family members for the presence of this mutation. A previously reported mutation (Pro437Leu) in exon 12 was found in a patient with Parkinson disease and essential tremors. This patient has relatives with Parkinson disease and essential tremors, which had not previously been reported in patients with this mutation. We are examining other family members to determine if this mutation is associated with both phenotypes. Our results suggest that the Pro437Leu mutation may predispose individuals to both Parkinson disease and essential tremors, and may shed light on the earlier controversy concerning association between these two common movement disorders.

**Hospital-based survey for family history in multiple system atrophy.** *Y. Momose<sup>1</sup>, J. Goto<sup>2</sup>, M. Murata<sup>2</sup>, S. Tsuji<sup>2</sup>.*  
1) Dept Clinical Bioinformatics, Grad Sch Med, Univ Tokyo, Tokyo, Japan; 2) Dept Neurology, Grad Sch Med, Univ Tokyo, Tokyo, Japan.

Multiple system atrophy (MSA) is an adult-onset, progressive neurodegenerative disorder characterized clinically by various combinations of autonomic failure, cerebellar symptoms, parkinsonism and pyramidal signs. MSA has been regarded to be a sporadic disease because familial occurrence has not been detected. Recent studies, however, suggest families in which multiple members were affected with pathologically-proven MSA. These studies raise the possibility that genetic factors are involved in the pathogenesis of MSA. To evaluate genetic components in MSA, we conducted a hospital-based survey on clinical genetics of MSA. Through the survey of consecutive medical records of patients who were admitted to University of Tokyo Hospital (1981 May 2003), we identified 87 patients with the clinical diagnosis of MSA (5 definite, 63 probable, and 19 possible cases) who satisfied the diagnostic criteria (except for the item of family history) for MSA formulated by the Consensus Committee of American Autonomic Society and the American Academy of Neurology. We identified a pedigree of familial MSA. There is no consanguinity. The proband was referred to our hospital because of progressive autonomic, cerebellar, pyramidal and extrapyramidal symptoms and his sister has also suffered from autonomic symptoms and parkinsonism refractory to l-dopa with MRI findings compatible with MSA. Although MSA has been regarded as a sporadic neurodegenerative disease, the present studies suggest involvement of genetic components in MSA. Identification of additional familial cases of MSA should provide important clues as to the pathogenetic mechanisms of MSA.

**Comprehensive analysis of candidate modifier genes for multiple sclerosis (MS) on chromosome 19q13.** *J.L. Haines<sup>1</sup>, S. Schmidt<sup>2</sup>, S. Sawcer<sup>3</sup>, L.F. Barcellos<sup>4</sup>, S.G. Gregory<sup>2</sup>, J. Sims<sup>2</sup>, M. Booze<sup>2</sup>, J.M. Vance<sup>2</sup>, J.R. Oksenberg<sup>4</sup>, S.L. Hauser<sup>4</sup>, A. Compston<sup>3</sup>, M.A. Pericak-Vance<sup>2</sup>.* 1) Vanderbilt University Medical Center, Nashville, TN; 2) Duke University Medical Center, Durham, NC; 3) Cambridge University, Cambridge, UK; 4) University of California, San Francisco, CA.

We previously reported an effect of the apolipoprotein E (APOE) locus on progression of MS, with the APOE-4 allele associated with a more severe disease course, and the APOE-2 allele associated with mild MS. While the APOE polymorphism may be the causal variant influencing MS expression, the results could also be due to linkage disequilibrium (LD) of APOE alleles with one or more causal variants in another gene. The APOE region harbors several other candidate genes for MS expression, including the poliovirus receptor (PVR), the poliovirus receptor-related gene (PRR2), and a gene coding for an outer mitochondrial membrane protein (TOMM40). To date, we have genotyped a total of 27 SNPs in a 1 Mb interval around APOE in an effort to identify the most likely functional variant. Association signals of similar strength ( $p=0.003$  to  $0.007$ ) with MS severity, but not MS risk, were observed for variants in TOMM40 and APOE, but not PVR and PRR2. To test this association and further resolve the localization, we genotyped an additional 780 MS parent-child trios evaluated using similar diagnostic criteria. Our combined data set of almost 1500 MS patients and their family members is the largest one available for examining MS modifier genes on chromosome 19q13. Preliminary data strengthen the association of mild MS with APOE-2 is ( $p=0.001$ ), but not the association of severe MS with APOE-4 ( $p=0.43$ ). Genotyping of TOMM40 and APOE promoter SNPs is in progress for additional single-locus and haplotype-based analyses.

**The serotonin transporter polymorphism, 5HTTLPR, is associated with a faster response time to sertraline in an elderly population with Major Depressive Disorder.** *S.M. Webb<sup>1</sup>, L.K. Durham<sup>2</sup>, P.M. Milos<sup>1</sup>, C.M. Clary<sup>3</sup>, A.B. Seymour<sup>1</sup>.* 1) Genomic and Proteomic Sciences, Pfizer, Groton, CT; 2) Nonclinical Biostatistics, Pfizer, Groton, CT; 3) Clinical Development, Pfizer, New York, NY.

A common polymorphism (5HTTLPR) within the promoter region of the serotonin transporter gene (SLC6A4) has been shown to influence response time to paroxetine in elderly depressed and overall response to fluoxetine in younger subjects with major depressive disorder (Pollock et al., 2000; Smeraldi et al., 1998). Based on these findings we hypothesized that a similar effect in response time to sertraline would be observed and that no effect would be seen in a placebo arm. We tested the hypothesis that subjects homozygous for the Long allele (LL) at 5HTTLPR would respond more rapidly to sertraline than subjects carrying one or two copies of the Short allele (SS/LS). LL subjects showed a significant increase in response at weeks 1 and 2 as assessed by the CGI-I scale compared with SS/LS subjects. No significant difference was observed in the placebo group. These results suggest that genetic variation in the serotonin transporter gene affects the response time to sertraline and provides complementing evidence to previous reports that this polymorphism affects response time to other SSRIs.

**Cohort and age of onset analysis of female breast cancer in non-Spanish surnamed Whites and three Asian ethnic groups in Los Angeles County, from 1975 to 1999.** *B.A. Corey<sup>1</sup>, J. Setyawati<sup>2</sup>, Y.F. Yu<sup>2</sup>, J.N. Weitzel<sup>1</sup>*. 1) Clinical Cancer Genetics, City of Hope Medical Center, Duarte, CA; 2) Univ of Southern California, School of Pharmacy, Dept. of Pharmaceutical Economics and Policy, Los Angeles, CA.

Rates of cancer are subject to cultural factors. As immigrant groups become assimilated to lifestyle in the United States their rates of breast cancer alter from that of their native lands. Of interest is the population of Asian women living in Los Angeles County who traditionally have low rates of breast cancer. Utilizing data collected on 115,436 subjects by the Cancer Surveillance Program of Los Angeles County from 1975 to 1999, we describe the epidemiology of female breast cancer in four population groups through cohort analysis and analysis of age at diagnosis. Cohort analysis was carried out in non-Spanish surnamed whites (NSSW) and 3 Asian ethnic groups: Chinese, Filipino and Japanese. Age cohorts were selected at 20-year intervals and their age-specific incidence of breast cancer was followed from 1975 to 1999. Age at diagnosis of ductal, lobular and medullary neoplasms and of all histologies were analyzed for proportion of breast cancers diagnosed prior to menopause. In cohort analysis, the youngest cohort of each group (age 45-49) showed increased incidence of breast cancer compared to women of the same age from the preceding cohort. Youngest cohorts of Japanese and Filipinos showed a faster rate of increase in breast cancer incidence relative to Chinese and were similar to NSSW. Proportion of diagnoses before 50 years of age was greatest in Chinese and Filipino women (> 40%), intermediate in Japanese women (28.5%) and lowest in NSSW women (19%). The data show increasing rates of breast cancer, and a higher proportion of early onset disease in Asians compared to NSSW, and suggest that mammography should be considered at an earlier age for Asian women. Supported (in part) by the National Cancer Institute Grant #R25 CA85771.

**Genotyping of colorectal cancer patients to genotypically define clinical outcomes and phenotypic features. E.**

*Friedman<sup>1,3</sup>, S. Starinsky<sup>1,3</sup>, R. Geva<sup>2</sup>, A. Figer<sup>2,3</sup>.* 1) Oncogenetics Unit, Inst Gen, Chaim Sheba Medical Ctr, Tel Hashomer, Israel; 2) The Oncology Institute, Elias Sourasky Medical Center, Tel-Aviv, Israel; 3) The Sackler School of Medicine, Tel-Aviv University, Ramat Aviv, Tel-Aviv, Israel.

Colorectal cancer (CRC) is the fourth most common cancer and the lifetime risk for developing CRC is 3-5% in the general population. Clinical outcome and therapeutic response is highly dependent on disease stage, tumor invasion, and tumor size and site. Yet it is likely that genetic factors play a major role in determining the clinical outcome and therapeutic response of CRC patients. In order to gain insight into this issue, we genotyped 456 consecutive Israeli patients with histopathologically confirmed CRC, who were unselected for family history of cancer, for 17 single nucleotide polymorphisms (SNPs) or missense mutations in 12 candidate genes: APC, beta-catenin, K-RAS, DCC, P16, PTEN, RB1, P15, APOE, ERCC2, P53, hMSH2. Correlation with disease outcome parameters, tumor location, stage and grade, was carried out by applying the chi test, determining the Hardy Weinberg equilibrium, Armitage trend test, and the Kaplan Meir test for survival. For the entire patient population, a statistically significant association between a SNP in the APC gene and age at diagnosis was shown ( $P_{\text{genotype}}=0.0595$ ), beta-catenin and APOE SNPs and the existence of a positive family history of cancer (beta-catenin:  $PHWE=0.013269$ ,  $P_{\text{genotype}}=0.034$ , APOE:  $P_{\text{genotype}}=0.033$ ), and P53 R72P mutation and overall survival ( $P_{\text{Kaplan Meier}}=0.0336$ ). In analysis of the Ashkenazim, phenotypic features that were significantly associated with a specific genotype: ERCC2 SNP and the C677T polymorphism in the MTHFR gene with age at diagnosis above or below 50 years (ERCC2:  $P_{\text{alleles}}=0.025$ ,  $P_{\text{Armitage trend}}=0.03$ , MTHFR:  $P_{\text{genotype}}=0.0005$ ); A P53 intron 3 polymorphism and APOE SNP, were significantly associated with having a family history of colon cancer (P53:  $P_{\text{alleles}}=0.022$ ,  $P_{\text{genotype}}=0.034$ ,  $P_{\text{Armitage trend}}=0.016$ , APOE:  $P_{\text{alleles}}=0.049$ ,  $p_{\text{genotype}}=0.04$ ). The results of this preliminary study show that genetic factors do play a role in determining CRC phenotype.

**Analysis of AGTR1 gene in a non-hypertension population sample from Amazon-Brazil.** *L. Dolinsky*<sup>1, 2</sup>, *S. Roig*<sup>3</sup>, *M. Boia*<sup>4</sup>, *P. Cabello*<sup>3</sup>, *R. Moura-Neto*<sup>1</sup>. 1) Dept Gen, Inst de Biologia, Univ Fed do Rio de Janeiro, Rio de Janeiro, Brazil ; 2) Dept Biociências, Unigranrio, Rio de Janeiro, Brazil; 3) Dept Gen, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil; 4) Medicina Tropical, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

Cardiovascular diseases are the main important public health problem in Brazil with almost 25% of the Brazilian population having high blood pressure and mortality rate around 32%. Due to the high incidence of cardiovascular diseases in Brazil we studied a gene (AGTR1) of the renin angiotensin system, one of the most important in the maintenance of cardiovascular homeostasis. AGTR1 has a genetic polymorphism (A1166C) which is associated with essential high blood pressure, especially in pregnant women. In this work we compared two different populations for AGTR1 polymorphism: one from the North of Brazil (Amazon), with no hypertension individuals, and a control population. Our goal here is to verify if there are allelic frequency differences between these two samples, excluding pregnant women. To analyse 100 individuals per population we used PCR reaction followed by DdeI digestion. Genotype and allelic frequency, and statistical analysis were done using Genetic Data Analysis software.

Populations showed no deviation from Hardy-Weinberg equilibrium, but showed significant differences between allelic frequencies ( $\chi^2= 9.17$ ,  $p=0.0062$ ). These data corroborate the notion that C allele polymorphism from AGTR1 gene is associated with high blood pressure even when pregnant women were excluded from the analysis.

**Combined tests of linkage and association of 4 SNPs in the FXIII A-subunit gene to FXIII activity levels.** *M. de Lange*<sup>1</sup>, *T. Andrew*<sup>1</sup>, *H. Snieder*<sup>1,3</sup>, *R.A.S. Ariëns*<sup>2</sup>, *M. Stickland*<sup>2</sup>, *P.J. Grant*<sup>2</sup>, *T.D. Spector*<sup>1</sup>. 1) Twin Research Unit, St Thomas' Hospital, London, UK; 2) Academic Unit of Molecular Vascular Medicine, Leeds General Infirmary, Leeds, UK; 3) Georgia Prevention Institute, Department of Pediatrics, Medical College Georgia, Augusta GA, USA.

**Background.** Activated factor (F) XIII cross-links fibrin and thereby enhances the mechanical strength of a clot and increases the resistance to fibrinolysis. The prevalence of a common point mutation in the FXIII A-gene (FXIII Val34Leu) was lower in patients with MI than those without, suggesting a protective role of this polymorphism. This linkage and association study using several SNPs within the FXIII A-subunit gene aims to locate the functional polymorphism.

**Methods.** A total of 201 dizygotic twin pairs were genotyped for 4 SNPs in the FXIII A-subunit gene (FXIII -246 G/A, FXIII Val34Leu, FXIII 564 C/T and FXIII 651 G/C) and used for tests of linkage, association and combined linkage and association using QTDT software.

**Results.** Significant linkage of FXIII activity was shown to the SNPs Val34Leu ( $p=5.10^{-12}$ ) and 651 G/C ( $p=0.03$ ), but not for -246 G/A ( $p=0.18$ ) or 564 C/T ( $p=0.09$ ). Also, significant association was shown for -246 G/A ( $p=4.10^{-6}$ ), Val34Leu ( $p=3.10^{-49}$ ) and 564 C/T ( $p=0.001$ ), but not for 651 G/C ( $p=0.72$ ). Linkage in the presence of association only showed a drop in fit for the FXIII Val34Leu polymorphism (from  $\chi^2=47.55$ ,  $p=5.10^{-12}$  to  $\chi^2=1.30$ ,  $p=0.25$ ), indicating that this locus is either the functional polymorphism or in strong linkage disequilibrium with the genetic variant. No drop in fit was observed for FXIII 651 G/C. No linkage or association was detected for any of the SNPs to levels of FXIII A-subunit or FXIII B-subunit.

**Conclusion.** The results of this linkage and association test of multiple SNPs in the FXIII A-subunit gene indicate that the FXIII Val34Leu polymorphism is (close to) the functional polymorphism influencing FXIII activity.

**Frequency of PvuII (LPL) genetic polymorphism in patients with Arterial Coronary Disease from Mexican population.** *L. Arnaud Jr<sup>1,2</sup>, M.P. Gallegos<sup>2</sup>, R.P. Mariaud<sup>1,2</sup>, L.J. Ramirez<sup>1,2</sup>, L.E. Figuera<sup>3</sup>, A.J. Ventura<sup>4</sup>, R. Gaxiola<sup>5</sup>, T.J. Beltran<sup>2</sup>, G.M. Zuniga<sup>2</sup>.* 1) Doctorado en Genetica Humana, UDG; 2) Division de Medicina Molecular, CIBO; 3) Division de Genetica Molecular, CIBO; 4) Servicio de Cardiologia; 5) Servicio de Epidemiologia, HE, CMNO, IMSS, Guadalajara, Jalisco.

**INTRODUCTION:** The Arterial Coronary Disease is the principal cause in morbidity and mortality in the world. It is considered a multifactorial disease. In the development of the ACD influence genetics and environmental factors. Between the risk factors most important is find abnormalities in the lipids metabolism whose provide correlation with ACD. Some studies have described the PvuII polymorphism association between gene Lipoprotein Lipase (LPL) and ACD, even so this association is contradictory in others parts of the world. This association is unknown in Mexican population. **OBJECTIVE:** To determine the genotypic frequency of PvuII polymorphism in the gene LPL in case-control study in Mexican population with Arterial Coronary Disease. **MATERIAL AND METHODS:** In this study, genomic DNA from 59 ACD patients and 46 controls were genotyped. Analysis of PvuII polymorphism was done by PCR amplification of a segment of 430 bp witch is localized in the intron 6 of the LPL gene in the chromosome 8. This has a recognition site for the PvuII restriction enzyme followed by PAGE at 6% and stained with silver nitrate. Genotypes were identified as A1/A1 (430bp), A2/A2 (218 and 212 bp). **RESULTS:** The presence of the A1/A1 genotype was identified in 43.5% of the controls and 17.8% of the patients with ACD, whereas 21.7% of the controls and 29.7% of the cases were homozygous A2/A2. Statistical difference was found when both group were compared ( $p < 0.05$ ). **CONCLUSIONS:** It was clear an increased frequency of allele A2 in patients group as in other parts of the world, this could demonstrate the contribution to the pathogenesis of the ACD.

**Association of apolipoprotein B signal peptide polymorphism in patients with myocardial infarction from**

**Mexican population.** *M. Gallegos Sr<sup>1</sup>, Y. Valdez<sup>1</sup>, L.E. Figuera<sup>2</sup>, A. Ventura<sup>3</sup>, F. Pérez<sup>3</sup>, T.J. Beltrán<sup>4</sup>, M.R. Arechavaleta<sup>5</sup>, L. Arnaud<sup>1</sup>, R.P. Mariaud<sup>1</sup>, G.M. Zuñiga<sup>1</sup>.* 1) División de Medicina Molecular, CIBO, IMSS; 2) División de Genética, CIBO, IMSS; 3) Departamento de Cardiología; 4) Departamento de Urgencias; 5) Departamento de Endocrinología, HE, CMNO, IMSS, Guadalajara, Jalisco, Mexico.

The three amino acid insertion/deletion (I/D) polymorphism in the apoB signal peptide (27 amino acids versus 24 amino acids signal peptide) was evaluated as a possible risk factor for myocardial infarction (MI) in a case-control study, population comprising 114 MI patients and 132 controls. In controls, homozygotes for the deletion allele (D/D) had the highest average levels of both total cholesterol and low density lipoprotein cholesterol, the homozygotes for the insertion allele (I/I) had the lowest average values, while the heterozygotes (I/D) had intermediate average levels. In MI patients, the trend was similar with no differences statistically significant were observed. However statistically differences in genotype frequencies (genotypes D/D in controls 0.09 and cases 0.29, I/I 0.42 in controls and 0.34 in cases and I/D 0.49 in controls and 0.37 in cases) were detected between cases and controls ( $p < 0.05$ ). Several studies have shown that the frequency of D/D is higher in hyperlipidemic than in normolipidemic groups. This marker is probably in linkage disequilibrium with some other atherogenic gene(s). Our study shows that differences, in both apoB signal peptide alleles and D/D and I/D genotypes distributions, are statistically significant ( $p < 0.05$ ). This marker had probably a predictive value in patients with IM in our populations.

**Heritability of Venous Function in Humans.** *A. Busjahn*<sup>1</sup>, *M. Brinsuk*<sup>2</sup>, *J. Tank*<sup>2</sup>, *F.C. Luft*<sup>2</sup>, *J. Jordan*<sup>2</sup>. 1) HealthTwiSt GmbH, Berlin, Germany; 2) Franz-Volhard Zentrum fuer Herz-Kreislaufforschung, Charité, Berlin, Germany.

**Introduction:** Venous function contributes to the pathogenesis of thrombophlebitis, venous thrombosis, and possibly to orthostatic intolerance. Venous disease is highly heritable; however, the genetic variance of venous function is unknown.

**Methods:** We determined the heritability of venous function in 46 twin pairs (24 monozygotic, age 35+/-11 yrs, 14 men, 34 women, and 22 dizygotic, age 30+/-8 yrs, 19 men, 25 women). All studies were conducted in the morning hours. After a resting phase in the supine position, we determined venous function in both legs by impedance plethysmography. Venous capacity was determined by a standardized protocol. In addition, we obtained venous pressure volume curves by slowly deflating a thigh cuff from 60 to 0 mmHg. Venous compliance was determined by linear regression analysis at the steepest part of the venous pressure volume curve. Heritability (h) was estimated using a path modeling approach.

**Results:** Venous capacity was 6+/-2.2 ml/100ml in monozygotic twins and 5.1+/-2.3 ml/100 ml in dizygotic twins. Venous compliance was 0.24+/-0.14 %/mmHg in monozygotic twins and 0.18+/-0.12 %/ mmHg in dizygotic twins. Unadjusted heritability (h<sup>2</sup>) was 0.6 (p<0.05) for venous capacity and 0.9 (p<0.05) for venous compliance. The heritability estimates remained essentially unchanged after adjustment for gender and age.

**Conclusions:** Venous function is strongly influenced by genetic factors. The genes involved may influence venous disease states. Moreover, they may contribute to variability in orthostatic tolerance. Currently we extend the dataset and initiate candidate gene studies.

**Association analysis of  $\alpha_2$ -adrenoceptor haplotypes with essential hypertension in White and Black Americans. X.**

*Bao*<sup>1</sup>, *A. Joyner*<sup>1</sup>, *P. Mills*<sup>3</sup>, *J. Dimsdale*<sup>3</sup>, *P. Cadman*<sup>2</sup>, *F. Rao*<sup>2</sup>, *B.K. Rana*<sup>3</sup>, *N. Schork*<sup>3</sup>, *D. O'Connor*<sup>2</sup>, *M. Ziegler*<sup>1</sup>. 1) Medicine, UCSD Medical Center, San Diego, CA; 2) Nephrology and Hypertension, UCSD VA Medical Center, San Diego, CA; 3) Psychiatry, UCSD Medical Center, San Diego, CA.

To investigate the relevance of genetic variants in  $\alpha_2$ -adrenergic receptor (ADRB2) to essential hypertension, 11 single-nucleotide polymorphisms (SNPs) in the promoter and coding region of ADRB2 gene were genotyped in an unrelated population (356 hypertensives, 665 normotensives). Using a maximum likelihood method, 12 haplotypes out of the theoretically  $2^{11}$  (=2048) possible combinations were found distributed divergently in Caucasian (n=659) and African-American (n=362) groups ( $p < 0.0001$ ). In the Caucasian group, three common haplotypes accounted for 96% of all haplotypes. They were analyzed as six haplotype pairs (diplotypes). African-Americans had more diversity in ADRB2 gene with five common haplotypes and 11 diplotypes accounting for 95% of all haplotypes. Mean lymphocytes  $\alpha_2$ -adrenergic receptor density and the low frequency spectrum of heart rate variability were significantly related to diplotype ( $P < 0.01$ ) but not to a single haplotype or individual SNPs in Caucasians. In both White and Black groups, the frequency of individual SNPs and haplotypes as well as diplotypes did not differ overall between the hypertensive and the normotensive. However, the diplotype frequency did differ by post hoc analysis among young Caucasians (age < 40,  $p < 0.05$ ). The diplotypes were related to a family history of hypertension in African-Americans ( $p < 0.05$ ). These results suggest important interactions of multiple SNPs within a haplotype and between haplotypes. We did not confirm prior reports of a relationship between individual SNPs and hypertension among Caucasians, but there may be a relationship between diplotype and hypertension among young Caucasians.  $\alpha_2$  adrenergic diplotype did relate to a family history of hypertension among African-Americans.

**High-throughput genotyping for identification of genes underlying coronary heart disease: Cumulative perspective from 222 polymorphisms in 111 candidate genes.** *J.J. McCarthy<sup>1</sup>, A. Parker<sup>2</sup>, D.J. Moliterno<sup>3</sup>, W.J. Rogers<sup>4</sup>, L.K. Newby<sup>5</sup>, R. Cannata<sup>3</sup>, K. Glatt<sup>2</sup>, E.J. Topol<sup>3</sup>, The GeneQuest Investigators.* 1) GSPH, San Diego State University, San Diego, CA; 2) Millennium Pharmaceuticals, Inc., Cambridge, MA; 3) Cleveland Clinic Foundation, Cleveland, OH; 4) University of Alabama Medical Center, Birmingham, AL; 5) Duke University, Durham, NC.

**BACKGROUND AND METHODS:** Widespread access to SNPs has facilitated genetic association studies of coronary artery disease (CHD) on a large scale. Here we present data for 222 common SNPs in 111 candidate genes typed in 352 Caucasians with familial, premature CHD (onset <45 males, <50 females) and 418 Caucasian population controls. Genotype frequencies were compared between cases and controls using logistic regression. **RESULTS:** SNPs in 30 genes previously associated with CHD were evaluated in our study and significant ( $p < 0.05$ ) associations were found in the following: APOE, CYBA, F7, FGB, GP1BA, IL1RN, MTHFR, F2, F5 and SELP. In addition, novel associations were found with SNPs in 13 genes: ANXA4, ECE1, HRG, LRP1, PAI2, PLCG1, PLOD2, PROC, SDC4, THBS1, THBS2, THBS4, THPO. The most significant CHD association was found with FGB R478K ( $p < 0.0005$ ), one of four tightly linked SNPs, along with 455G/A, -655G/A and S189S, in the Beta Fibrinogen gene for which genetic associations with CHD have been previously described. Individuals who carried the K allele were at decreased odds of CHD (OR 0.59; 95% CI 0.41, 0.85), even after adjusting for age, gender, diabetes, hypertension and body mass index and population stratification. Restricting the cases to those with MI enhanced many associations, the strongest being with THBS4 A387P ( $p < 0.004$ ) where presence of the variant allele increased odds of MI. We also identified a region of strong linkage disequilibrium on 1q22-q25 that spans a number of candidate genes including F5, SELP and SELL. **CONCLUSIONS:** Despite concerns regarding false positives from multiple testing, and false negatives, from incomplete assessment of SNPs, large-scale association analysis can be an effective means of hypothesis generation and offers a complementary strategy to classic linkage studies of the genetic basis of CHD.

**A Comprehensive Analysis of a Missense Mutation in the Rho-kinase Gene that Potentially Causes Coronary Vasospastic Ischemic Heart Disease in the Japanese.** *H. Kamiunten, G. Koike, J. Mashiba, H. Shimokawa, A. Takeshita.* Cardiovascular medicine, Kyushu University, Fukuoka, Fukuoka, Japan.

**Background** Coronary vasospasm is one of the major causes of ischemic heart disease (IHD) and its prevalence is known to be higher in the Japanese than that in the western population. We have shown that Rho-kinase (also known as ROCK2) plays a crucial role in coronary vasospasm in humans. However, the genetic impact of Rho-kinase gene variation on coronary vasospasm remains to be examined. **Methods and Results** We have recently identified a novel single nucleotide polymorphism (SNP), G930T (K310N), in the catalytic domain of Rho-kinase. Japanese patients with IHD (n=215) and Caucasian ones from the ENCORE I trial (n=318) were genotyped for the Rho-kinase SNP and also for additional 4 polymorphisms of interest, including ACE I/D, angiotensinogen C704T, angiotensin II type 1 receptor A1166C, and eNOS C-786T polymorphisms in each population. The Japanese patients were classified into 3 groups based on their coronary responses to intracoronary acetylcholine administration; large epicardial coronary artery spasm group (LAS), microvascular spasm group (MVS), and normal group (N). The frequency of the T930 allele is higher in the LAS+MVS group (4/111 for the LAS group and 2/53 for the MVS group) than in the N group (0/51) and the general population (1/98,  $p=0.008$ ) in the Japanese. Interestingly, no Caucasian patients had the T930 allele. There was no remarkable association between coronary vasospasm and the other 4 polymorphisms. Human coronary artery smooth muscle cells were infected with adenoviruses expressing the wild type (G930) or the mutant (T930) Rho-kinase. Western blot analysis revealed no increase in phosphorylation level of ERM in cells infected with the mutant Rho-kinase compared to that in cells infected with the wild type. The similar result was observed in F-actin staining. **Conclusions** The G930T variation of Rho-kinase have a significant association with coronary vasospasm in the Japanese. The functional analyses indicated that the mutant Rho-kinase may induce hyperresponse to vasoconstrictive stimuli resulting in coronary vasospasm. Further analysis is now ongoing.

**Contribution of a promoter polymorphism in the AGTR1 gene to Hypertension in African Americans and Latinos in Los Angeles, California.** *S. Henderson*<sup>1,2</sup>, *C. Haiman*<sup>2</sup>, *W. Mack*<sup>2</sup>. 1) Department of Emergency Medicine, Keck School of Medicine of the University of Southern California, Los Angeles, CA; 2) Dept. of Preventive Medicine, Keck School of Medicine of the University of Southern California, Los Angeles, CA.

**OBJECTIVE:** African Americans have excess hypertension (HTN), and its associated complications, when compared to other U.S. populations, and this excess is particularly significant in younger individuals. The Renin-Angiotensin-Aldosterone system (RAAS) is critical to the maintenance of blood pressure and genetic variants in the Angiotensin II Type 1 Receptor (AGTR1) genes have been associated with the risk of HTN in some populations. We theorized that a polymorphism in the AGTR1 gene would contribute to HTN risk in AAs when compared to another racial-ethnic group with a similar socioeconomic status, specifically Latinos. **METHODS:** We genotyped 1252 African American and 1042 Latino members of a large multiethnic cohort to determine the frequency of the C(-535)T variant in the AGTR1 gene, and its association with HTN. **RESULTS:** There was a significant increase in risk of HTN for African Americans associated with the AGTR1 T allele (OR =2.62 (95%CI: 1.46,4.72) for the heterozygote and OR =2.67 (95%CI: 1.51,4.74) for the homozygote:  $p = 0.03$  for the trend). No such trend was seen among Latinos (OR=0.91 (95%CI: 0.68,1.20) for the heterozygote and 1.31 (95%CI: 0.78,2.18) for the homozygote,  $p=0.79$  for the trend). **CONCLUSION:** There is a significant risk of hypertension associated with the (-535)T allele of the AGTR1 (-535) gene in the AA population, a finding not seen in the Latino population.

**Genetic and clinical factors in PR pediatric patients with thromboembolic disease (TED) at the UPR Pediatric Hospital.** *N.I. Lopez-Molina<sup>1</sup>, J.Y. Renta<sup>1</sup>, M.A. Ayala<sup>1</sup>, A. Rivera<sup>1</sup>, I. Rivera<sup>1</sup>, J.R. McFaline<sup>1</sup>, P.J. Santiago-Borrero<sup>2</sup>, C.L. Cadilla<sup>1</sup>.* 1) Biochemistry Department, UPR School of Medicine, San Juan, PR; 2) Pediatrics Department, Hereditary Diseases Program, UPR School of Medicine, San Juan, PR.

Many studies have demonstrated a relatively high prevalence of thromboembolic disorders (TED) in neonates and children, especially in very-low-birth weight newborns and very sick children. These clinical findings have been associated with deficiencies of antithrombin, proteins C and S and plasminogen; and the presence of common mutations in the prothrombin (G20210A), factor V (G1691A, Leiden), and the methyltetrahydrofolate reductase (MTHFR, C677T) genes. We studied twenty seven (27) Puerto Rican (PR) TED pediatric patients between the ages of one (1) and nineteen (19) years that were treated at the Thromboembolic Clinic of the UPR Pediatric Hospital, both or one of their parents and twenty-two (22) control subjects, to determine the role of genetic or environmental factors in TED. The TED patients had at least one thromboembolic event confirmed by imaging studies and were not on anticoagulation treatment at the time of the thromboembolic event. The control patients had the same age range as the TED patients, and were chosen at random at the Pediatric Emergency Clinic. We found eight (8) carriers of the MTHFR C677T among 18 TED patients. Nine out of 19 controls carried the MTHFR C677T mutation, and three were homozygous for the mutation. The MTHFR carrier frequencies in TED patients and control subjects compare well with previous studies on 400 PR newborns where the carrier frequency for the C677T mutation was found to be ~50%, and the frequency of homozygotes was 14%. None of the TED patients had FV Leiden alleles. In 122 PR newborns the allele frequency of the FV Leiden mutation was 0.41%. These data suggest that the MTHFR C677T mutation is not more frequently found among PR children with TED, and that the FV Leiden mutation is not frequently found among Puerto Ricans. This study was supported in part by grants from RCMI G12RR03051, MBRS RISE R26GM61838 and SCORE S06GM08224.

**Analysis of ACE gene in a non-hypertension population sample from Amazon-Brazil.** *S.R.S. Roig<sup>1</sup>, L. Dolinsky<sup>2, 3</sup>, M. Boia<sup>4</sup>, R. Moura-Neto<sup>2</sup>, P. Cabello<sup>1</sup>.* 1) Genetica, Fundacao Oswaldo Cruz, Rio de Janeiro, Brazil; 2) Genetica, Univ Fed Rio de Jan, Rio de Janeiro, Brazil; 3) Biociencias, Unigranrio, Rio de Janeiro, Brazil; 4) Medicina Tropical, Fundacao Oswaldo Cruz, Rio de Janeiro, Brazil.

High blood pressure affects almost 10 million Brazilian people and it is an important risk factor for cardiovascular diseases. Hypertension etiology involves both genetics and environmental factors. The Renin-Angiotensin System (RAS) plays a role in the physiology of vascular diseases, and genes involved in this system may be related to hypertension development. Actually exists many reports demonstrating the association of renin-angiotensin system gene polymorphism with blood pressure regulation in different populations. In order to test this association, we analyzed the angiotensin I converting enzyme (ACE) D/I gene polymorphism in two different populations from Brazil. We performed an association study with this polymorphism in 100 individuals from Amazon and a control population. Genotyping was performed using PCR reaction. Allelic frequencies and statistical analysis were done using Genetic Data Analysis software.

Allelic frequencies showed significative differences between the two analyzed populations ( $X^2=71.77$ ,  $p=0.00$ ). These data corroborate that I allele is associated with normal to low blood pressure.

**Pleiotropic effect of genes influencing hemostasis phenotypes on type 2 diabetes status.** *D. Warren<sup>1</sup>, J.M. Soria<sup>2</sup>, J.C. Souto<sup>2</sup>, J. Fontcuberta<sup>2</sup>, J. Blangero<sup>1</sup>, L. Almasy<sup>1</sup>.* 1) Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

Type 2 diabetes is a complex disease influenced by multiple genes and environmental factors, both of which are largely unknown. Hypercoagulation often occurs in diabetic patients, suggesting possible pleiotropy of genes influencing both hemostasis-related phenotypes and diabetes status. These pleiotropic effects may contribute to the increased risk of thrombosis, myocardial infarction, retinopathy, and nephropathy observed in diabetics. Whether increased clotting in diabetics occurs because of hyperactivity of procoagulants, depressed anticoagulant or fibrinolytic activity, or a combination of factors, is unclear. To better understand the mechanisms behind hypercoagulation in diabetics, we used bivariate variance-components methods to examine the correlation between diabetes status and 16 quantitative hemostasis-related traits in randomly ascertained Mexican Americans participating in the San Antonio Family Heart Study. Data were available for 811 subjects from 39 extended families. 155 diabetics were identified by WHO criteria or previous diagnosis. Quantitative traits examined include clotting factors II, V, VII, VIII, X, XI, and XII, activated protein C ratio (APCR), activated partial thromboplastin time (APTT), free and total protein S (fPS & tPS), protein C, prothrombin time, tissue factor pathway inhibitor (TFPI), thrombin activable fibrinolysis inhibitor, and von Willebrand factor (vWF). Additional covariates include age, sex, and exogenous hormone use. Analyses indicate positive genetic correlations (0.38-0.51,  $p < 0.05$ ) between diabetes status and levels of proteins that enhance (factors II & VIII, vWF) and inhibit (tPS, TFPI) coagulation. Two measures of hypercoagulability, APTT and APCR, correlate negatively (-0.38 & -0.49,  $p < 0.05$ ) with diabetes status. Our results suggest that genes influencing hemostasis-related traits pleiotropically influence risk of diabetes. Although both pro- and anticoagulant levels are elevated in diabetics, the hypercoagulability observed in this disease suggests genes that promote clotting have the greater impact on disease phenotype.

**Heritability associated with candidate polymorphisms for cardiovascular risk factors.** *M.-H. Roy-Gagnon<sup>1,2</sup>, S.H. Jee<sup>3</sup>, A.F. Wilson<sup>1</sup>*. 1) Genometrics Section, IDRB, NHGRI, NIH, Baltimore, MD; 2) Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) Department of Epidemiology and Health Promotion, Graduate School of Public Health, Yonsei University, Seoul, Korea.

Quantitative traits, such as plasma levels of coagulation factors, may determine risk factors for cardiovascular disease (CVD). Understanding the genetic mechanisms underlying these traits may help to unravel the complex etiology of CVD. In this study, the contribution of known candidate polymorphisms to the heritability of CVD-related traits is estimated. The Regression of Offspring on Mid-Parent (ROMP) approach was used to investigate heritability in 87 Korean families (508 individuals) of probands who underwent elective coronary arteriography. ROMP is an extension of the traditional regression of offspring on mid-parent used to estimate trait heritability. ROMP also provides a test of association and an estimate of the heritability attributable to a candidate locus. Total and locus-specific heritability estimates were obtained for 14 traits including coagulation factors VII and fibrinogen, plasminogen activator inhibitor - 1 (PAI-1), total and HDL cholesterol. Five candidate polymorphisms for PAI-1, factor VII and fibrinogen were considered. Estimates were adjusted for age, sex, smoking and alcohol drinking. Parent-of-origin effects were also investigated. Estimates of total heritability for factor VII, PAI-1, HDL cholesterol and triglyceride levels were significant ( $p < 0.0001$ ) and ranged from 45 to 66%. The estimated heritability of fibrinogen levels was 14 10%. Three linked fibrinogen polymorphisms were associated with fibrinogen and PAI-1 levels (locus-specific heritability estimates of 1 0.3% and 3 1%, respectively;  $p < 0.015$ ). Clotting factor VII was associated with the factor VII polymorphism with a locus-specific heritability estimate of 2 0.8% ( $p = 0.01$ ). These polymorphisms account for only a small percentage of the heritability of the traits (3-7%). This suggests that several other genes and/or shared familial and environmental factors may be responsible for the high heritability of these traits.

**COMPLEX SEGREGATION ANALYSIS OF BLOOD PRESSURE IN THREE MIDDLE DALMATIAN ISLANDS, CROATIA.** *T. Skaric-Juric*<sup>1</sup>, *E. Ginsburg*<sup>2</sup>, *E. Kobyliansky*<sup>2</sup>, *I. Malkin*<sup>2</sup>, *N. Smolej Narancic*<sup>1</sup>, *M. Pericic*<sup>1</sup>, *P. Rudan*<sup>1</sup>. 1) Institute for Anthropological Research, Zagreb, Croatia; 2) Department of Anatomy and Anthropology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Complex segregation analyses of systolic and diastolic blood pressure have been performed on family data from the populations of the island of Brac, Korcula and Vis (Middle Dalmatia, Croatia), separately, with the purpose to elucidate a potentially promising population for the search of the genes responsible for high prevalence of hypertension. According to the criteria for developed hypertension (systolic BP $\geq$ 160 and/or diastolic BP $\geq$ 95 mm Hg), the prevalence ranged from 31% on Brac, to 38% on Korcula and 41% on Vis (36% for all islands). Complex segregation analysis has been performed by implementation of the program package MAN. The sample encompassed 928 individuals, aged 17-85 yr. (490 from Brac, 282 from Korcula and 156 from Vis). By applying the usual transmission probability tests, the major gene (MG) hypothesis for systolic and for diastolic blood pressure has been accepted only in the sample from the island of Vis. For both blood pressures, the major gene as well as environmental hypotheses have been rejected in the sample from Korcula, while on the sample from Brac, along with the rejection of MG model, the environmental model - indicating action of polygenetic and environmental family factors - could not be rejected. According to the estimates revealed from the non-constrained MG model, in the sample from Vis, the proportion of (age and sex adjusted) variance of blood pressure that could be attributed to the effect of a hypothetical major gene (H<sub>2</sub>) is 41.0% for systolic and 42.3% for diastolic blood pressure. The respective values for total genetic variance (h<sup>2</sup>) are 76.5% and 59.7%. High prevalence of hypertension combined with the results of the complex segregation analyses of blood pressure in three isolated Middle Dalmatia islands point to the island of Vis, as a potentially promising target population for incoming molecular genetic studies of hypertension.

**Study of the Isolated Populations in the National Park of Cilento and Vallo di Diano and Comunita Montana**

**Ufita.** *M. Persico*<sup>1</sup>, *M. Ciullo*<sup>2</sup>, *V. Colonna*<sup>1</sup>, *M. Astore*<sup>1</sup>, *A. Calabria*<sup>1</sup>, *T. Nutile*<sup>1</sup>, *W. Longo*<sup>1</sup>, *A. Fierro*<sup>2</sup>, *R. Pacente*<sup>1</sup>, *G. Antoniol*<sup>3</sup>, *J. Guardiola*<sup>1</sup>. 1) IGB A. Buzzati Traverso, CNR, Napoli, Italy; 2) BioGeM s.c. a r.l., Ariano Irpino (Av), Italy; 3) Research Centre on Software Technology, Benevento, Italy.

One approach useful to identify loci and genes responsible for complex diseases takes advantage of populations of small villages that have been geographically isolated for centuries. We looked at the villages on the hills and mountains of the National Park of Cilento e Vallo di Diano and of the Comunita Montana Ufita, two areas in Southern Italy. We chose some of these villages on the basis of a history of high endogamy (marriages between people born in the same village). In the Comunita Montana Ufita, we have chosen the village of Montaguto where the collection of anagraphic data is in progress. In the Cilento region, we have already collected the last three centuries genealogical data of the population in three villages, Campora, Cardile and Gioi, in order to build their genealogy. These villages have at the present a population of about 600/800 habitants. All genealogical informations have been stored in a relational database. Although the villages are located in the same area, historical data report a different origin of these populations. Moreover, the plague in Southern Italy in the 1656 strongly hit these villages determining a population bottleneck. Computational analysis of genealogical male and female hereditary lines was carried out to determine the ancestral villages founders. This analysis allowed to identify 51 unrelated males and 48 unrelated females. Further, the analysis of mitochondrial (mtDNA) and Y chromosomal DNA performed on these individuals allowed a further reduction of the number of hypothetical founders. In order to establish the homogeneity of the population we are evaluating the extent of linkage disequilibrium (LD) by the analysis of a number of microsatellites in chromosome region Xq13.3. We will present preliminary data on the health of the population of Campora village: clinical analyses of blood samples, medical interviews and examination.

**Influence of vascular endothelial growth factor gene polymorphisms on cardiac defects in 22q11 deletion syndrome.** *E.J. Seo<sup>1,2</sup>, J.J. Lee<sup>1</sup>, S.M. Park<sup>1</sup>, J.Y. Yang<sup>1</sup>, Y.H. Kim<sup>1,3</sup>, J.K. Ko<sup>1,3</sup>, H.W. Yoo<sup>1,3</sup>, S.J. Kim<sup>4</sup>, H.B. Oh<sup>2</sup>, K.J. Lee<sup>5</sup>, I.S. Park<sup>1,3</sup>.* 1) Genome Research Ctr for BDGD, Asan Medical Center, Seoul, Korea; 2) Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea; 3) Department of Pediatrics, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea; 4) Department of Pediatrics, Sejong Hospital, Seoul, Korea; 5) Department of Statistics, Dongguk University.

The phenotype of 22q11 deletion syndrome is extremely various among del22q patients, even within same family. Cardiac defects are found in 75% of 22q11 deletion syndrome. Recent report has shown that vascular endothelial growth factor (VEGF) gene is a modifier of cardiovascular birth defects in this syndrome. VEGF is a potent regulator of vasculogenesis and angiogenesis. Aim of the present study was to determine whether the VEGF gene polymorphisms are associated with cardiac defects in 22q11 deletion syndrome. We examined five common polymorphisms in the promoter region, 5'UTR and 3'UTR of the VEGF gene in 63 patients with 22q11 deletion, 22 families of 22q11 deletion, 53 control subjects and 16 control families. Allele frequencies of -2578C>A, -1498T>C, -1154G>A, -634C>G and 936C>T in controls were 0.314, 0.276, 0.245, 0.546, and 0.093, respectively. Any genotype distribution did not differ significantly between del22q patients with and without cardiac defects, and between controls and del22q patients with cardiac defects. Association studies were performed in families of del22q patients with cardiac defects and control families using transmission disequilibrium test (TDT) and haplotype based haplotype relative risk (HHRR) statistics. TDT analysis showed no evidence of preferential transmission of any VEGF alleles studied. We did not identify the important associations between VEGF gene polymorphisms and cardiac defects in del22q patients, which might be caused by limited number of subjects. Extended study for larger populations will be required to confirm that VEGF gene increase the risk for cardiac defects in 22q11 deletion .

**Unexpected discordance of lung disease severity between monozygous twins with cystic fibrosis.** *G.R. Cutting<sup>1</sup>, R. McWilliams<sup>1</sup>, J. Hoover-Fong<sup>1</sup>, S. Beck<sup>2</sup>, K. Naughton<sup>1</sup>, C. Yurk<sup>1</sup>, C. Gruver<sup>1</sup>, P. Cornwall<sup>1</sup>, D. Fallin<sup>1</sup>, A. Hamosh<sup>1</sup>.*  
1) Johns Hopkins Univ, Balt, MD; 2) St. Chris Hosp for Children, Phila, PA.

To identify factors independent of the disease-causing gene that contribute to cystic fibrosis (CF) phenotype variability, we have undertaken a multicenter study of CF-affected twins. Thus far, blood samples, clinical and environmental data have been collected from 25 monozygous (MZ) twin pairs and 14 dizygous (DZ) twin pairs. Five sets of twins were excluded in this analysis (3 DZ sets of opposite sex, 1 DZ and 1 MZ set with a member with other significant medical problems) yielding 13MZ/8DZ male pairs and 11MZ/2DZ female pairs. All DZ and 22 MZ pairs are both alive, 1 MZ pair are both dead, and 1 MZ pair is discordant for vital status. There were no differences between MZ and DZ pregnancies in gestational age, complications or mode of delivery. Similar portions of MZ (74%) and DZ (80%) twins were homozygous for F508, the common CF mutation. Concordance was assessed using Fishers Exact Test for categorical data and within pair differences were examined using t-tests and ANOVA for continuous data. As expected, MZ twins were more concordant than DZ twins for height at diagnosis (within twin diff: MZ 1.00.8 cm, DZ 4.63.2 cm;  $p < 0.02$ ), current height, and meconium ileus (MZ 23 concordant/1 discordant, DZ 9/4;  $p < 0.04$ ). Unexpectedly, MZ twins were no more concordant than DZ twins for measures of lung disease severity (current FEV<sub>1</sub>, FVC, and latest chest X-Ray). Furthermore, inspection of longitudinal pulmonary function tests on 17 MZ pairs and 7 DZ pairs revealed 3 MZ pairs who had notably different lung function throughout their lives. Despite sharing similar environments, MZ and DZ twins were discordant for pathogens that commonly infect CF patients (*P. aeruginosa* and *B.cepacia*). Interestingly, MZ twins had greater exposure than DZ twins to environmental agents likely to affect lung disease progression, including wood stoves, passive smoke, cockroaches, rodents and history of exercise. Taken together, these data indicate that environmental and stochastic factors play a substantial role in variation of lung disease, the major cause of morbidity and mortality in CF patients.

**Haplotype analysis suggests the wild-type allele is a modifier of the severity of the nail dysplasia in Nail Patella Syndrome.** *J. Dunston*<sup>1</sup>, *J.W. Park*<sup>1,2</sup>, *M. Malbroux*<sup>1</sup>, *I. McIntosh*<sup>1</sup>. 1) Inst Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 2) Dept Epidemiol. Bloomberg School of Public Health, Johns Hopkins Univ, Baltimore, MD.

Nail Patella Syndrome (NPS) results from haploinsufficiency of the LMX1B gene, which codes for a LIM-homeodomain transcription factor. NPS is typically characterized by nail dysplasia, elbow dysplasia, absent/hypoplastic patellae and exostoses of the ilia. Nail dysplasia decreases in severity from thumb to fifth finger and is variable in severity both within and between families. Previous genetic evidence suggested that the allele inherited from the unaffected parent might influence the severity of the nail dysplasia. To test this hypothesis, we applied a quantitative scoring system to the nail dysplasia in 89 individuals from 41 families with NPS. Mutations in LMX1B have been identified in 40/41 families. Possible nail scores range from 0 to 6.32, with 0 representing no nail changes and 6.32 representing the complete absence of all nails. The observed scores ranged from 0 to 5.70 with a mean of 2.32 and standard deviation of 1.06. The presence or absence of the creases over the distal interphalangeal (DIP) joint was noted; 93 % of individuals were missing 1 or more creases. Neither age, sex, mutation type, absence of DIP joint creases or sex of the parent from whom the disease-causing mutation was inherited is a predictor of the nail score. To test the effect of the LMX1B allele inherited from the unaffected parent, six SNPs, including two newly identified SNPs, were genotyped across the LMX1B gene. Haplotypes were constructed using family data to set phase. For singletons, haplotypes were predicted using the population frequencies. Haplotypes with a population frequency greater than 0.05 were included in subsequent analyses. Simple linear regression was used to test the total effect of each haplotype on the nail score. Two of six haplotypes gave a significant result. One haplotype appears to be protective ( $\beta=-0.93$ ,  $p=0.041$ ) and the other appears to increase risk ( $\beta=0.93$ ,  $p=0.041$ ), supporting the hypothesis that the wild-type LMX1B allele is a modifier of this aspect of the NPS phenotype.

**Polymorphisms of the C677T and A1298C of the MTHFR gene in Alzheimer's diseases.** *I. Kara<sup>1</sup>, E. Eker<sup>2</sup>, T. Ertan<sup>2</sup>, F. Engin<sup>2</sup>, A. Sazci<sup>3</sup>, E. Ergul<sup>3</sup>, G. Akpinar<sup>3</sup>, G. Kaya<sup>1</sup>.* 1) Department of Neuroscience, Institute for Experimental Medical Research, Istanbul University, Istanbul, Turkey; 2) Department of Psychiatry, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey; 3) Department of Medical Biology and Genetics, Faculty of Medicine, University of Kocaeli, Kocaeli, Turkey.

Alzheimers disease (AD) is a progressive neurodegenerative disorder of the central nervous system and resulting in dementia in more than 65 years old individuals. Although a number of genes has been shown involved in familial and sporadic AD, mutations in these particular genes are responsible for only 1 percent of all AD patients. The methylenetetrahydrofolate reductase (MTHFR) gene has been suggested to be a candidate gene for AD. The MTHFR C677T and A1298C polymorphisms may cause hyperhomocysteinemia by reducing 5-methyltetrahydrofolate availability. The aim of the study was to investigate the contribution of MTHFR polymorphisms; C677T and A1298C to AD. A PCR-RFLP method was used in the analysis of the genotypes followed by a statistical analysis. We examined 94 AD patients (mean age 72.76 plus minus 10.44) and 225 healthy control subjects (mean age 70.39 plus minus 11.99). Clinical diagnosis of AD patients was made according to the NINCDS-ADRDA and DSM-IV criteria. The allelic frequencies of the MTHFR T677 and MTHFR C1298 were 34.57 percent; and 31.38 percent; in the AD patients and 34 percent; and 29.11 percent; in the controls respectively. The allelic distribution was not significant in the MTHFR T677 ( $\chi^2=0.614$ ;  $P=0.736$ ) whereas it was significant in the MTHFR C1298 ( $\chi^2=7.588$ ;  $P=0.023$ ). Frequencies for MTHFR C677C, C677T and T677T were 39.4 percent; 52.1 percent; and 8.5 percent; in the AD patients and 42.2 percent; 47.6 percent; and 10.2 percent; in the control subjects respectively. Likewise, frequencies for MTHFR A1298A, A1298C and C1298C were 41.5 percent; 54.3 percent; and 4.3 percent in the AD patients and 51.6 percent; 38.7 percent; and 9.8 percent in the controls respectively. The MTHFR A1298C genotype showed increased risk for AD ( $OR=1.881$ ; 95 percent  $CI=1.157-3.060$ ;  $\chi^2=6.564$ ;  $df:1$ ;  $P=0.010$ ). The MTHFR C677C/A1298A compound genotype was protective for AD ( $OR=0.397$ ; 95 percent  $CI=0.160-0.981$ ;  $\chi^2=4.240$ ;  $df:1$ ;  $P=0.039$ ). In conclusion, the MTHFR A1298C genotype is associated with AD.

**Autism: Incidence of Cytogenetic abnormality and Fragile-X syndrome.** *K. Reddy, The Diagnostics Team.*  
Genzyme Genetics, Orange, CA.

A total of 434 patients with an indication of autism were referred for genetic diagnosis. The median age was 4.1 years. Sex ratio was 4.4 males to 1 female. Fragile-X testing (Southern blot & PCR) found 6/316 [1.89%] with abnormal results. The mutations were: A full mutation (fM) and abnormal methylation in 3 [50%] and mosaic mutations with partial methylation of variable clinical significance in 3 [50%]. The mosaic mutations were: a fM [200-900 repeats(r)]/deletion mutation [30 r] with partial methylation, 2 size mosaics had permutation (pM) [150 r]/fM [400 r], and pM [155 r]/fM [800 r] with normal and abnormal methylation. A premutation mosaic female carrier with atypical EcoR1 and Eag1 pattern and a typical BssH1 pattern gave 2.8, 3.0, 5.2-5.4 Kb bands. PCR gave reproducible bands corresponding to 29, 65, 80 repeats and a faint band for 39 repeats. This probably indicates somatic mosaicism. Family studies may provide insight into the clinical implication. A Chromosome (cs) abnormality was found in 14/421 [3.33 %] cases. The aberrations were: 4/14 [28%] supernumerary markers from cs15 [3] and cs2 [1]; 4/14 [28%] deletions of 2q37.3, 3q25, 12q21.2q23.3 and 13q13.2q14.1; 1/14 [7%] duplication of 15q11.2q13; 3/14 [21%] inversions of 10p11.2q21.2, 17q23q25 (de novo) and 14q11.2q33 (mosaic); 2/14 [14%] translocations, one balanced t(1;14) and one unbalanced der(14;18). A cs15 was involved in 4/14 [28%] rearrangements (2 de novo & 1 mosaic), of which 3/4 had an extra copy/copies of D15S11 & GABRB3 and a 2q37 deletion support the association of autism with duplication 15q11.2q13 and in some cases 2q37 deletion. The frequency of a cs abnormality (3.33%) and FRAXA (1.89%) are at the lower end of other reported surveys (cs 2.8-11.5%, FRAXA 2-4%). The limitations of this study are: the retrospective nature of our study and absence of defined clinical criteria. Conclusions: when the indication is autism a higher incidence of mosaic FRAXA mutations with partial methylation [50% vs 15-20% in FRAXA patients] suggests faint bands and atypical Southern band pattern may be anticipated. The partial methylation may be the cause of deviant features like autism. Since 28% of the cs abnormalities were subtle, high resolution analysis is indicated.

**Evidence supporting the MFTM in autism.** *C.M. Wolpert<sup>1</sup>, J. Grubber<sup>1</sup>, S.L. Donnelly<sup>1</sup>, G.R. DeLong<sup>1</sup>, S.A. Ravan<sup>2</sup>, R.A. Abramson<sup>2</sup>, H.H. Wright<sup>2</sup>, J.R. Gilbert<sup>1</sup>, M.L. Cuccaro<sup>1</sup>, M.A. Pericak-Vance<sup>1</sup>.* 1) Duke Univ Medical Ctr, Durham, NC; 2) Univ of South Carolina, Columbia, S.C.

The genetic basis of autism is complex, with a sex difference in incidence (male:female ratio 4:1). Applying the multifactorial threshold model to autism we thus hypothesize that females would have a higher genetic load than males. Impaired adaptive behavior and age at onset (AAO) can both be used as indicators of severity. We analyzed a data set (N=234; Fhx- = no family history of autism (N=139); Fhx+; = family history of autism (N=95)) ascertained for genetic studies. All individuals met diagnostic criteria for autism as confirmed by the Autism Diagnostic Interview-Revised (ADI-R). The Vineland Adaptive Behavior Scales (VABS) was used to assess adaptive functioning. Lower VABS scores reflect greater severity. Between group comparisons (Mann-Whitney U-test) showed females (n=56) had lower communication (p=0.03) and adaptive behavior composite (ABC; p=0.03) scores than males (n=178). The data set was then stratified by family history status. Females from Fhx- families (n=28) had significantly lower scores than males from Fhx- families (n=111) on communication (p=0.002), daily living skills (p=0.013) and ABC (p= 0.009) scores while no gender differences were noted in Fhx+ families. No difference was noted between Fhx- and Fhx+ groups when collapsed across gender. ADI-R items 5 and 94 measure AAO. Earlier AAO is an indicator of greater severity. Between group comparisons revealed no gender differences on either item. However, when stratified by family history status, Fhx- females showed a significantly earlier AAO than Fhx- males (item 5, Chi-square p-value =0.03; item 94, Wilcoxon p-value=0.05). No differences were noted in Fhx+ families. Finally, no AAO difference was noted between Fhx- and Fhx+ groups when collapsed across gender. In summary, females from Fhx- families show an earlier AAO and more impaired adaptive behaviors. We believe these indices may have etiologic relevance in autism in isolated or sporadic cases of autism. The current findings suggest a complex relationship between severity, gender, and family history status in individuals with autism.

**Discovering haplotype blocks in the human genome.** *A. Rinaldo<sup>1</sup>, B. Devlin<sup>2</sup>, L. Wasserman<sup>1</sup>, K. Roeder<sup>1</sup>.* 1) Dept of Statistics, Carnegie Mellon University, Pittsburgh, PA; 2) Dept of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Haplotype blocks are segments of chromosomes that apparently have been transmitted through many generations without experiencing substantial effects of recombination (i.e., segments behave as blocks). The identification of haplotype blocks can substantially reduce the cost of finding genetic variants associated with increased risk of disease by decreasing the number of polymorphisms required to screen genomic segments. In this work we provide a statistical characterization of haplotype blocks and develop a methodology to identify them. The algorithm possesses two main features: (1) Shannon's entropy is used as a measure of multivariate correlation among SNPs to locate groups of consecutive SNPs that are likely to belong to the same block; and (2) the physical boundaries of the blocks are found by implementing a model selection procedure that estimates the penalty for model complexity based on biological justification. The methodology performs well when evaluated by simulated data. Results obtained by using data from the UW-FHCRC Variation Discovery Resource (SeattleSNPs) indicate that the regions found by our procedure exhibit the expected characteristics of a haplotype block.

**Estimating main effects and interactions of genes through a structured stepwise dimension reduction (SSDR) of measures of association.** *N. Wang, R. Chakraborty.* Center for Genome Information, University of Cincinnati, Cincinnati, OH.

Recent literature supports the notion that identification of all components of genetic control of complex disease phenotypes is difficult without that a structured strategy of multipoint/multilocus linkage/association analysis. This is intimately connected with the complexity of phenotypes, which makes their familial resemblance deviate from the predictions of principles of simple Mendelian rules. As a consequence, deconvolution of multilocus genotype data into haplotypes, and/or deciphering multilocus/multi-site association results into a minimal set of markers, which explain the association results, constitutes a critical component of identifying the main effects as well as interaction of the genetic factors controlling any complex phenotype. Using classical measures of association applied to data of case-control study designs, we used a structured stepwise dimension reduction (SSDR) technique to attribute an overall significant association to a minimal set of classes, which in turn detects main effects as well as interactions of genes that can explain an observed phenotype-genotype association. This method is more general than the traditional haplotype inference methods, since the different sites of the SSDR method do not necessarily have to be genetically linked. Thus, the SSDR technique is argued to be helpful in determining gene-gene interactions, useful in studying gene ontology relationships. When the sites belong to linkage groups, this technique can also infer the antiquity of haplotypes and the history of populations in which they are observed. Through applications of such SSDR techniques, we show that phenotypes such as susceptibility to food allergies may be under genetic control through interactions of genes, rather than main effects of individual genetic polymorphisms. In addition, an application of SSDR to two sets of haplotype data from multiple populations is shown to be consistent with the Out-of-Africa origin of modern humans. (Research supported by US Public Health Service Research grants).

**A Significant Association between Nonsyndromic Oral Clefts and Arylhydrocarbon Receptor Nuclear Translocator (*ARNT*).** K. Kanno<sup>1,2</sup>, S. Kayano<sup>1,2</sup>, A. Yamada<sup>1</sup>, S. Kure<sup>2</sup>, Y. Suzuki<sup>2</sup>, Y. Matsubara<sup>2</sup>. 1) Plastic/Reconstructive Surg, Tohoku Univ School of Med, Sendai, Miyagi, Japan; 2) Dept. Medical Genetics, Tohoku Univ School of Med, Sendai, Miyagi, Japan.

The etiology of nonsyndromic oral clefts (cleft lip; cleft palate; or cleft lip and palate) 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) found in tobacco, herbicides, contaminated food; TCDD administered during organogenesis in mice causes a high incidence of cleft palate in fetuses. Aryl hydrocarbon receptor (AHR), aryl hydrocarbon receptor nuclear translocator (*ARNT*), and cytochrome P450 1A1 (*CYP1A1*) are involved in TCDD metabolism, and it is noteworthy that AHR, *ARNT* and *CYP1A1* mRNA is expressed during the craniofacial tissue development of mice and human fetuses. When TCDD was administered to pregnant mice, almost all wild-type fetuses suffered from cleft palate, whereas none of the AHR-null mutant fetuses were sensitive to the teratogenic effects of TCDD. This evidence suggests that genes *AHR*, *ARNT*, and *CYP1A1* may play a role in nonsyndromic oral clefts. In the present study, we assessed whether there is any association in the Japanese population of nonsyndromic oral clefts and single nucleotide polymorphisms (SNPs) in *AHR*, *ARNT*, and *CYP1A1* using the transmission disequilibrium test (TDT). We investigated SNPs in *AHR*(1661A/G), *ARNT*(567G/C, IVS12-19T/G), and *CYP1A1*(1384A/G), which were identified in Japanese. The SNPs were subjected to TDT for 148 patient-parents trios. The C allele of *ARNT* 567G/C was preferentially transmitted to patients ( $P=0.0334$ ). When *ARNT* haplotypes 567G/C and IVS12-19T/G were simultaneously considered, the preferential transmission of the CT (567C-IVS12-19T) haplotype was significant ( $P=0.0012$ ). Although the GG haplotype was transmitted to patients less than 50% of the time, the significance of this deviation was marginally significant ( $P=0.052$ ). Deviation of transmission rates for the other SNPs was not observed. Our results suggest that *ARNT* is involved in the development of nonsyndromic oral clefts in the Japanese population.

**Inherited Pathway for Exceptional Longevity.** *G. Atzmon<sup>1</sup>, P. Rabizadeh<sup>1</sup>, R. Gottesman<sup>1</sup>, A.R. Shuldiner<sup>2</sup>, N. Barzilai<sup>1</sup>.* 1) Institute for Aging Research, Dept Medicine, AECOM, New York, NY; 2) University of Maryland School of Medicine, and the Geriatrics Research and Education Clinical Center, Baltimore, MD.

Subjects with exceptional longevity have a lower incidence of age-related disease, and their family members may inherit biological factors that modulate aging processes and disease susceptibility. To identify specific genetic factors that predict the human longevity phenotype, we recruited Ashkenazi Jewish probands with exceptional longevity (n = 213; age 98.2±5.3 years), their offspring (n=242; age 68.3±6.7 years) and age-matched control group of Ashkenazi Jews (n=258). The odds ratio of reaching over the age of 90 was 6.9 in the proband's parent generation compared to controls (P<0.001), supporting the role of genetics in their longevity. The frequencies of only three SNPs in 3 candidate genes for cardiovascular disease (*Br J Haematol.* 114(3):718-20), were significantly different in long-lived probands compared to the ~70 year old control group. In particular, homozygosity for the 405 valine (V) allele of cholesteryl ester transfer protein (CETP) (VV genotype) was 25% in the proband group and 20% in the offspring group compared with only 5% in the control group (p<0.001 vs. proband and offspring). Those with the VV genotype also had ~30% lower serum CETP concentrations. Because CETP is involved in regulation of lipoprotein and their particle sizes, we determined these (by NMR technology), and found that HDL and LDL particle sizes were significantly larger in probands and their offspring compared with age-matched controls. Moreover, in CETP VV homozygotes, HDL and LDL particle sizes were significantly larger in offspring without hypertension, and cardiovascular disease (p<0.008), and in probands with better cognitive function (p<0.05). We conclude that the V allele of CETP has pleiotropic effects on lipoprotein and other cardiovascular traits as well as cognitive function that protect from cardiovascular disease and cognitive decline, and promote exceptional longevity in humans.

Program Nr: 1184 from 2003 ASHG Annual Meeting

**Epidemiology and Genetics of Duchenne muscular dystrophy in Northern Ireland.** *K. McKeever, A. Magee.*  
Regional Genetics Service, Belfast City Hospital Trust, Belfast, N Ireland, United Kingdom.

**Objective;** Creation of a database of affected males and their first degree relatives. This will facilitate study of the epidemiology and natural history of this devastating neuro-muscular disease and allow completion of molecular investigations for each pedigree. Carrier females have a significant risk of developing cardiac complications including cardiomyopathy, therefore identification of this group is imperative.**Methods;** Review of patient notes held in the department. Notes were identified with the use of a database, GENESIS, and with the help of the charity, Muscular Dystrophy Campaign.**Results;** Since 1980, 123 cases of Duchenne muscular dystrophy (DMD) have been diagnosed. There are 59 males and 3 manifesting carriers alive and resident in Northern Ireland. The prevalence is 3.5 per 100,000 population and incidence is 18.2 per 100,000 live births i.e. 1 in 5492 live births. The average age of surviving males is 12.21 years with a range of 3.3-26.1 years. The percentage survival at age 20 is 57.1% and mean life expectancy 17.42 years. Thirty-six (61%) of the current cases are sporadic and 23 (39%) are inherited. Thirty-five (59%) of the mutations are due to deletions of one or more exons; another 2 are contiguous gene deletions and there is 1 insertion. One further point mutation has been identified in muscle. In 20 cases the mutation is as yet unidentified. There are 23 confirmed carrier mothers from the population of 59 males and 17 mothers whose status is unknown. We have identified 93 other female first degree relatives of all cases of DMD identified since 1980 (both alive and deceased) whose carrier status is unknown; the family mutation is known in 46 (49%). **Conclusions;** We have created a database of Duchenne muscular dystrophy families in Northern Ireland. This has identified at risk females who can now be offered genetic counselling, testing, and cardiac assessment where appropriate. The survival of affected males at age 20 in Northern Ireland has now passed 50% creating a significant population in Adolescent and Adult Medicine.

**Allelic frequencies of nine loci STR in a sample of Bolivar City.** *J.A. Nastasi, A. Parrilla, H.O. Guzmán, A. Rodríguez, M. Suegart, G. Partidas, L. Eduard, M. López, O. Sánchez.* Unidad de Genética, Centro de Microscopía Electrónica, Esc. de Ciencias de la Salud, Universidad de Oriente, Ciudad Bolívar, Estado Bolívar, Venezuela.

The study of DNA polymorphism is extremely useful to anthropological and forensic sciences. The loci STR are extensively used because of their high informativity, heterozygosity, and easy reproducibility, and it has been preferred as a molecular tool in several areas of medicine and biology. We analyzed nine tetranucleotide loci STR highly informative in a sample of Bolivar City, Venezuela. The typing was performed by polymerase chain reaction (PCR), according with the conditions of the technical manual of GenePrint STR System of Promega. Denaturant polyacrilamide gel (6%) was used to resolve the amplified fragments of PCR, and reveled by silver staining technique. We calculated the allelic frequency of CSF1PO, TPOX, TH01, F13A01, FESFPS, vWA, F13B and LPL, as STR autosomic loci, and the locus HPRTB localized in X chromosome. We established allelic frequencies to our city, for every one of nine loci above mentioned, and these frequencies were compared with other published similar studies. Our allelic frequency are slight different to other Caucasian, American, African and Asian populations previously reported, but have higher similarity with other Latin-American populations. The matching probability including all nine loci STR studied for our population was  $5,42 \times 10^{-8}$ , and the typical paternity index to nine loci mentioned above was 184,5 and finally, the power of exclusion to all loci was 0,99759997. These results would be use in forensic casework and anthropological studies.

**Low cancer incidence rates in Ohio Amish.** *J.A. Westman, S.N. MacEachern, A.K. Ferketich, S. Lemeshow, R. Pilarski, R. Nagy, A. de la Chapelle, C.D. Bloomfield.* The Ohio State University Comprehensive Cancer Center, Columbus, OH.

Little is known about cancer in the Amish. The Amish of Holmes County, Ohio and neighboring areas is a unique founder population in north central Ohio and the largest Amish community in the world. The cancer incidence rate among the Ohio Amish was estimated for the reference years 1996-1999. A random sample of 92 households was interviewed and a detailed four- to five-generation cancer family history taken, resulting in a database of 27,580 unique individuals, with 26,679 living during the reference years. A total of 545 cancer diagnoses were documented with 108 diagnosed during the reference years, 35 of whom are still living. The population in the reference years had 18,201 adults over age 20 (9103 females and 9098 males) with 105 cancer occurrences in adults. Diagnoses were confirmed when possible with medical records, death certificates, and comparison to the state of Ohio cancer incidence surveillance system. The total person-time contribution to each of seven age categories was computed and crude rates estimated and compared to that seen in the general Ohio population. The cancer cases were classified using the National Cancer Institutes Surveillance, Epidemiology and End Results (SEER) classifications. An age-adjusted rate was computed for all cancer types, colon, breast, and prostate cancers in the Amish and compared to the Ohio rate. Age-adjusted rates were not computed for less common cancer types but the observed number of cases were compared with the expected number of cases for that age group. The age-adjusted cancer incidence rate for all cancers among the Amish was estimated to be  $192.2/10^5$  person-years (Ohio  $643.6/10^5$  person-years;  $Z = 12.99$ ;  $p < 0.0001$ ), or 30% of the general population incidence. The incidence of every SEER cancer type was lower in the Amish than in the general population. Lifestyle factors, generally considered responsible for 60% of cancer occurrences, are not likely to be the sole contributors to the observed reduction in cancer incidence since reduced tobacco and alcohol use is offset by increased obesity. Protective genetic factors may be present that reduce cancer susceptibility in the Ohio Amish.

**Large scale DNA variation as an aid to reconstruction of extended human pedigrees.** *S.R. Woodward<sup>1, 3</sup>, N. Myres<sup>3</sup>, J.B. Ekins<sup>3</sup>, J.E. Ekins<sup>3</sup>, K. Hadley<sup>3</sup>, L. Hutchison<sup>2</sup>, L. Layton<sup>3</sup>, U. Perego<sup>3</sup>, A. Sims<sup>3</sup>, A. Nelson<sup>3</sup>, M. Nelson<sup>3</sup>, A. Welch<sup>3</sup>.* 1) Microbiology and Molecular Biology, Brigham Young University, Provo, UT; 2) Computer Science, Brigham Young University, Provo, UT; 3) Sorenson Molecular Genealogy Foundation, Salt Lake City, UT.

Reconstruction of pedigree structure from limited information is frequently required in studies of inherited diseases. To aid in this process, we have developed a database of genetic variation based on autosomal, mitochondrial, and Y chromosome DNA sequence. We have sampled a total of 37000 individuals of diverse ethnic origin, with an initial focus on European and Polynesian lineages verified by extended written genealogies. From 14000 of these individuals, we have typed 14 X chromosome microsatellites, 120 other autosomal microsatellites, 24 Y-chromosome microsatellites for more than 2.2 million locus tests. Markers were chosen in sets of 3 to 5 that are in tight disequilibrium and are distributed across the genome. In addition, we have sequenced from a subset of these samples 850 base pairs from the mitochondrial D-loop. The combination of genetic and genealogical data is maintained and managed in a mySQL based database from which input files for analysis programs including STRUCTURE and ARELIQUIN are generated. In addition, other analysis programs developed by us, YTYPEFINDER and HAPLOTYPYPER have been employed to generate modal haplotypes from the Y-chromosome data and to reconstruct phase in the X chromosome dataset where haplotyping algorithms can be assessed. We have employed the STRUCTURE program and additional analysis with HAPLOTYPYPER and YTYPEFINDER to measure genetic relationships across living individuals, and correlated these relationships with historical genealogical records. These analyses indicate that in Polynesian populations, clear assignment of living individuals to distinct historical lineages is feasible over the range of 3 to 6 generations, and similar assignments may be possible for living individuals in European populations.

**MTHFR polymorphism in patients with vascular dementia.** *E. L. Eker<sup>1</sup>, T. Ertan<sup>1</sup>, F. Engin<sup>1</sup>, A. Sazci<sup>2</sup>, E. Ergul<sup>2</sup>, G. Akpınar<sup>2</sup>, G. Kaya<sup>3</sup>, I. Kara<sup>3</sup>.* 1) Department of Psychiatry, Istanbul University, Istanbul, Turkey; 2) Department of Medical Biology and Genetics, Faculty of Medicine, Kocaeli University, 41900, Derince, Kocaeli, Turkey; 3) Department of Neuroscience, Institute for Experimental Medical Research, Istanbul University, Istanbul, Turkey.

Vascular dementia (VD) is the second most common form of dementia, following AD worldwide. VD is associated with a number of risk factors including MTHFR polymorphisms, resulting in hyperhomocysteinemia. MTHFR is an important risk factor for stroke. In the present study, MTHFR C677T and A1298C polymorphisms were examined as genetic risk factors for VD. Sixty-one patients (mean age 78.63 plus minus 8.78) with vascular dementia by the NINDS-AIREN criteria and 225 healthy control subjects (mean age 70.39 plus minus 11.99) were genotyped for MTHFR C677T and MTHFR A1298C polymorphisms using PCR-RFLP. The MTHFR T677 allele frequency was 36.07 percent in the VD patients and 34 percent in the control subjects. Frequencies for MTHFR C677C, C677T, and T677T were 32.8 percent, 62.3 percent and 4.9 percent in the VD patients and 42.2 percent, 47.6 percent and 10.2 percent in the control subjects. The allelic distribution by MTHFR C677T was insignificant ( $\chi^2=4.604$ ;  $df:2$ ;  $P=0.100$ ). The MTHFR C1298 allele frequency was 27.87 percent in the VD patients and 29.11 percent in the control subjects. Frequencies for MTHFR A1298A, A1298C, and C1298C were 50.8 percent, 42.6 percent and 6.6 percent in the VD patients and 51.6 percent, 38.7 percent and 9.8 percent in the control subjects. The allelic distribution of MTHFR A1298C was insignificant ( $\chi^2=0.743$ ;  $df:2$ ;  $P=0.690$ ). The MTHFR C677T genotype showed increased risk for VD (OR=1.822; 95 percent CI=1.020-3.255;  $\chi^2=4.171$ ;  $df:1$ ;  $P=0.041$ ). In conclusion, the MTHFR C677T genotype is a genetic risk factor for VD.

**Allele frequencies of 41 SNPs in 10 candidate genes for osteoporosis and obesity in a large Caucasian sample and comparison with the data from public databases and other studies.** *V. Dvornyk<sup>1</sup>, J.R. Long<sup>1</sup>, D.H. Xiong<sup>1</sup>, H.W. Deng<sup>1,2</sup>.* 1) Osteoporosis Research Center and Department of Biomedical Sciences, Creighton University, Omaha, NE; 2) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan , P. R. China.

SNPs have become increasingly used in gene identification studies for complex traits. However, accuracy and suitability of SNP data for studies of some complex disorders, such as osteoporosis and obesity, have often not been validated. We analyzed allele frequencies of 41 SNPs of ten candidate genes for osteoporosis and/or obesity in a large US Caucasian sample (1,873 individuals of both genders) by PCR-invader assay and maximum likelihood method. Out of 41 SNPs studied, 13 were firstly reported for Caucasians and eight of them were monomorphic. A comparison of the other 28 SNP allele frequencies of the studied Caucasian population with the respective data for Caucasians from the literature and public databases revealed significant difference for six SNP markers. Further comparison of our data with other ethnic groups confirmed the commonly observed differences in SNP allele frequencies for the three major world ethnic groups: 3 of 19 loci differed significantly in Caucasians vs Africans, and so do 13 of 24 loci in Caucasians vs Asians. Significant differences in allele frequencies of three polymorphisms (out of five compared) were also observed between Caucasian and Pacific Rim samples. The results of this extensive study essentially confirmed the previously reported data on the SNPs for Caucasians, and suggested several new candidate SNPs for studying osteoporosis and obesity. The observed significant differences in allele frequencies of some SNPs in Caucasians may be due to population admixture and/or insufficient sample size in the other studies. Our results also showed that major ethnic groups are indeed highly differentiated at some SNP markers of a number of important genes, which may be associated with osteoporosis and/or obesity. This differentiation may underlie different rates of these disorders in the major ethnic groups.

**Defining haplotypes and tagging efficient SNPs for association studies of candidate genes and regions for Cleft Lip and Palate.** *T. Beaty*<sup>1</sup>, *M.D. Fallin*<sup>1</sup>, *J.B. Hetmanski*<sup>1</sup>, *R. Ingersoll*<sup>2</sup>, *I. McIntosh*<sup>2</sup>, *A.F. Scott*<sup>2</sup>. 1) Dept Epidemiology, Johns Hopkins University, School of Public Health, Baltimore, MD; 2) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

Defining haplotype blocks and tagging SNPs that can identify these blocks has been proposed as one way to maximize the efficiency of genotyping for association studies while extracting the most genetic information. Characterizing haplotype structure should help identify the major variation in a candidate gene or region, so linkage disequilibrium information can be used to its greatest potential. Once haplotype structure is identified, only those SNPs that uniquely categorize chromosomes into common haplotypes need to be genotyped. This could substantially reduce the cost of a study while maximizing genetic association information. Before this strategy can be used for a particular candidate gene or region, however, haplotype blocks must be defined and SNP tags identified that best represent those blocks. We present analysis for 150 SNPs located in 27 genes and 2 extended chromosome regions (4p16, 22q11.21) selected as plausible candidates for cleft lip and/or palate. We first identified SNPs via sequencing and database searches, then genotyped SNPs in 145 unrelated Caucasian parents of cleft children. The number of SNPs identified per gene/region ranged from 1-30, spanning distances ranging from .05 kb 1.33 Mb. Analysis of allele frequencies, patterns of pair-wise linkage disequilibrium, haplotype block predictions and choices of tagging SNPs that would reconstruct the common haplotypes are presented. On average, only half of the original SNPs identified were needed for efficient typing. To contrast the set of informative SNPs for genotyping of these sample candidate genes in another population, the same strategy of defining haplotype blocks is being applied to an Asian control sample.

**Associations between Polymorphisms in the Steroid 5-Reductase Type II (SRD5A2) Gene and Benign Prostatic Hyperplasia and Prostate Cancer.** *M.T. Salam*<sup>1</sup>, *G. Ursin*<sup>1,2</sup>, *E.C. Skinner*<sup>3</sup>, *T. Dessissa*<sup>4</sup>, *J.K. Reichardt*<sup>1,4</sup>. 1) Preventive Medicine, University of Southern California, Los Angeles, CA; 2) Institute for Nutrition Research, University of Oslo, Norway; 3) Urology, University of Southern California, Los Angeles, CA; 4) Biochemistry & Molecular Biology, University of Southern California, Los Angeles, CA.

Genes affecting androgen synthesis and metabolism have been suggested to be involved in the development of both benign prostatic hyperplasia (BPH) and prostate cancer. We evaluated associations between three polymorphisms in the steroid 5-reductase type-II (SRD5A2) gene [A49T (alanine-49 to threonine), V89L (valine-89 to leucine) and a (TA)<sub>n</sub> dinucleotide repeat in the 3' UTR (untranslated region)] and both BPH and prostate cancer within a multiethnic population. Men between 60 and 86 years of age were recruited from annual prostate cancer screening programs and from a large urology clinic. We genotyped 606 men (412 Hispanic, 98 Caucasian, 73 African-American and 23 Asian) of whom 100 had prostate cancer, 393 had BPH (280 symptomatic and 113 asymptomatic) and 113 had normal prostates. Unconditional logistic regression was used to calculate the odds ratio (OR) and 95% confidence interval (CI) while adjusting for age and race. Overall, there was an association between V89L and BPH, with an OR for the LL (polymorphic) vs. VV (normal) genotype ( $OR_{LL/VV}$ ) of 1.9 (CI, 0.8-4.9,  $P_{trend} = 0.73$ ). This association was strongest in Hispanics ( $OR_{LL/VV} = 3.9$ ; CI, 0.9-17.3,  $P_{trend} = 0.03$ ). V89L was also associated with prostate cancer; the  $OR_{LL/VV}$  was 4.5 (CI, 1.2-16.1,  $P_{trend} = 0.01$ ) in the overall population and 7.3 in Hispanics (CI, 1.5-35.5,  $P_{trend} = 0.005$ ). Presence of at least one T allele at the A49T loci was associated with increased risk of BPH (OR = 4.5; CI, 0.6-37.0) and prostate cancer (OR = 4.1; CI, 0.4-49.0). These results suggest that the SRD5A2 gene may play an important role in both BPH and prostate cancer. Supported by CRP grant 99-000553V-10150.

**Multivariate genetic analysis of thyroid hormone traits.** *G. Perez<sup>1</sup>, N. Gouin<sup>2</sup>, C. Kammerer<sup>1</sup>, D. Finegold<sup>3</sup>, T.P. Foley<sup>3</sup>, S. Cole<sup>2</sup>, J. Blangero<sup>2</sup>, L. Almasy<sup>2</sup>, P. Samollow<sup>2</sup>, M.M. Barmada<sup>1</sup>.* 1) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 2) Southwest Foundation for Biomedical Research, San Antonio, TX; 3) Department of Endocrinology, UPMC Health System, Pittsburgh, PA.

Thyroid hormones play major roles in the regulation of protein synthesis, basal metabolism, and lipid metabolism. In an effort to understand the genetic and environmental influences which contribute to variation in these hormones, we have conducted multivariate modeling, and report here biologically meaningful latent factors with high heritability. We evaluated variation in seven thyroid hormone or related traits (free and total thyroxine (T<sub>4</sub>), free and total triiodothyronine (T<sub>3</sub>), thyroxine-binding globulin (TBG), thyrotropin (TSH), and thyroglobulin (TG)). Phenotype and genotype data were obtained from members of 33 randomly selected Mexican-American families from the San Antonio Family Heart Study. Multivariate linear regression was used to correct for environmental components of variation, and the resulting residuals were analyzed for correlation and for higher-order structure using factor analysis. Similar analyses were conducted with the inclusion of other hormones, lipids, indicators of fat mass, and insulin related measures, and also individually for sex-specific subgroups. For all factor models, regression-derived factor scores were generated for each factor and subjected to variance component modeling. Significant heritabilities (>30%) were observed for most factors, so that genome-wide linkage analysis was subsequently undertaken. Linkage signals (LODs ranging from 2.0-2.5) were identified across most models for a factor representing the relationship between TSH, T<sub>3</sub>, and T<sub>4</sub> levels at a locus on chromosome 4, near albumin, which is associated with Euthyroid Hyperthyroxinemia - a disease that manifests with abnormal total T<sub>4</sub> and T<sub>3</sub> levels. This signal was not identified by separate univariate QTL analyses of the thyroid traits. These results illustrate that analyses of multivariate traits will enhance the identification of possible pleiotropic QTLs that influence thyroid hormone function.

**Association of the hCLCA1 gene with bronchial asthma.** *F. Kamada*<sup>1</sup>, *Y. Suzuki*<sup>1</sup>, *C. Shao*<sup>1,3</sup>, *M. Tamari*<sup>2</sup>, *K. Hasegawa*<sup>2</sup>, *Y. Aoki*<sup>1</sup>, *S. Kure*<sup>1</sup>, *G. Tamura*<sup>3</sup>, *T. Shirakawa*<sup>2,4</sup>, *Y. Matsubara*<sup>1</sup>. 1) Department of Medical Genetics, Tohoku University School of Medicine, Sendai, Miyagi, Japan; 2) Laboratory for Genetics of Allergic Diseases, SNP Research Center, The Institute of Physical and Chemical Research, Yokohama, Japan; 3) Department of Respiratory and Infectious Diseases, Tohoku University School of Medicine, Sendai, Japan; 4) Department of Health Promotion and Human Behavior, Kyoto University School of Public Health, Kyoto, Japan.

Bronchial asthma is a chronic illness characterized by reversible airway obstruction and airway hyperresponsiveness that are caused by bronchial inflammation. This inflammation involves mucus overproduction and hypersecretion. Recently, a mouse model of asthma showed that *Gob-5* is involved in the pathogenesis of asthma. The observation suggests that the human homologue of *Gob-5*, *hCLCA1*, may be involved in the human disease. We screened for SNPs in the *hCLCA1* gene in the Japanese population to examine whether the *hCLCA1* gene polymorphisms are associated with asthma. We identified 8 SNPs (intron 5; 908+246C>T, intron 5; 908+2199G>A, intron 6; 1086+589T>C, intron 6; 1087754C>A, intron 6; 1087705A>G, intron 8; 1533+997A>C, intron 8; 1534808G>C, exon 15; 2787T>C) in this gene, and performed association studies using 1091 asthma patients (562 childhood patients, 529 adult patients) and 1105 non asthmatic controls. Association of 2787T>C with childhood asthma (P=0.025) and that of 1533+997A>C with adult asthma (P=0.026) were detected in the single-SNP case control study. In the 8-SNP haplotype analysis, we found a different haplotype distribution pattern between controls and childhood asthma (P=0.000089). The TGCCAAGT haplotype was protective against childhood asthma (P=0.016). In the diplotype analysis, individuals who had this haplotype showed lower risk for childhood asthma when compared to those who did not (P=0.013). Our data are the first to suggest the association of the *hCLCA1* gene polymorphisms and human asthma.

**Environmental and genetic etiology factors in cleft lip and palate in Korea.** *S. Jee*<sup>1</sup>, *B. Park*<sup>2</sup>, *M. Kim*<sup>1</sup>. 1) Graduate School of Public Health , Yonsei Univ, Seoul, Korea; 2) Department of Plastic and Reconstructive Surgery, Yonsei University College of Medicine, Seoul, Korea.

This study was an investigation by questionnaire, from January 1997 to July 2002, of 453 people who had been diagnosed and treated with cleft lip and/or palate in one hospital. Among them, 107 participated in the study. According to priorities, we investigated the kinds of cleft etiologies considered by their parents. The analysis showed that the first priority was drug intake during early pregnancy, and the second was paternal smoking. This study showed 13.1% (14 people) heritable cases in clefts. Furthermore, among those with and without a family history of clefts, drug intake during early pregnancy was selected as the first priority risk factor. However, among those cases with a family history, parental genetic etiology was selected for the second and third priorities, while among those cases without a family history, parental smoking, environmental factors, and orofacial clefts were selected. Unfortunately, little is known regarding the etiology and embryology of these clefts in the Korean population. Especially, we cannot disregard the effect of genetic interaction in clefts in considering the 13.1% family heritable cases in this study. It is evident from the results of this study that further investigations into the environmental and genetic etiology in the Korean population are required.

Program Nr: 1195 from 2003 ASHG Annual Meeting

**Evidence of gene-gene interaction as a strong risk factor for myocardial infarction: Combined carrier status of prothrombin 20210A and factor XIII-A L34 alleles.** *Y.G. Xie, C. Butt, H. Zheng, D. Robb, P. Parfery, E. Randell.* Memorial University of Newfoundland, Disciplines of Laboratory Medicine, Genetics, Pediatrics, Medicine, St. Johns, NL, Canada. A1B 3V6.

Conflicting results have been obtained from studies associating the prothrombin 20210A (FII 20210A), Factor V Leiden (FVL), and Factor XIII L34 (FXIII-A L34) alleles with myocardial infarction (MI). These conflicting results may result from complicated gene-gene interactions in the pathogenesis of MI, inadequate sample size, heterogeneous genetic background of subject populations and focusing only on the role of individual genetic variants. These weaknesses could be overcome through simultaneous analysis of multiple gene variants in a large sample size from the genetically isolated population. We have genotyped 600 MI patients and 500 controls from this genetically isolated Newfoundland population to determine the association between the FII 20210A, FVL and FXIII-A L34 alleles and MI. We also focused on the analysis of possible gene-gene interactions involved in the pathogenesis of MI in this population. The prevalence of the FII 20210A allele was higher in MI patients (2.8%) than in controls (1.0%;  $P=0.031$ ). The FII 20210A allele was also 5.1 fold higher in MI patients younger than 55 years when compared with age-matched controls ( $P=0.009$ ). Furthermore, the prevalence of combined carriers of FXIII-A L34 and FII 20210A alleles was 13-fold higher in MI patients compared with controls ( $P = 0.002$ ) with 93% penetrance. There was disequilibrium of the FXIII-A L34 allele in MI patients carrying the FII 20210A allele as a genetic background. From these observations, we conclude that: 1) the FII 20210A allele is a risk factor for MI, possibly important for early onset; 2) interaction between the FII 20210A and FXIII-A L34 alleles form a synergistic co-effect which strongly predisposes for MI, placing combined carriers at high risk for MI. We are currently collecting and analyzing further patient samples, focusing on those under the age of fifty-five years.

**Diversity of paternal and maternal lineages in populations of South Siberia.** *M. Derenko*<sup>1</sup>, *B. Malyarchuk*<sup>1</sup>, *T. Grzybowski*<sup>2</sup>, *A. Lunkina*<sup>1</sup>, *I. Dambueva*<sup>3</sup>, *F. Luzina*<sup>4</sup>, *D. Miscicka-Sliwka*<sup>2</sup>, *I. Zakharov*<sup>5</sup>. 1) Institute of Biological Problems of the North, Magadan, Russia; 2) The Ludwik Rydygier Medical University in Bydgoszcz, Bydgoszcz, Poland; 3) Institute of General and Experimental Biology, Ulan-Ude, Russia; 4) Institute of Professional Diseases and Hygiene Problems, Novokuznetsk, Russia; 5) Vavilov Institute of General Genetics, Moscow, Russia.

Phylogeography of the non-recombining uniparentally inherited mitochondrial DNA (mtDNA) and Y-chromosome has been broadly used to shed light on different aspects of genetic history of human populations. To investigate the origin and evolution of aboriginal populations of South Siberia, a comprehensive mtDNA (HVR1 or HVR1/HVR2 sequencing combined with RFLP typing of informative coding region polymorphisms) and Y-chromosome binary markers (YAP, RPS4Y, SRY-8299, M89, 12f2, M9, M20, 92R7, SRY-1532, DYS199, LLY22g, Tat) analysis of 1538 individuals representing 15 different populations (Koryaks, Evens, Evenks, Yakuts, Tofalars, Tadjiks, Tuvinians, Khakassians, Altaians, Teleuts, Shors, Sojots, Buryats, Mongolians, Koreans) was performed. The phylogenetic networks based on obtained data were further investigated following the phylogeographic analysis of individual lineage clusters. The total sample revealed 81% of East Asian (M\*, M7, M8, M9, M10, C, D, G, Z, A, B, F, N9a, Y) and 17% of West Eurasian (R\*, HV, H, U, J, T, I, N1a, X) matrilineal genetic contribution, but with regional differences. The highest influx of West Eurasian mtDNAs was observed in populations of Altai region of South Siberia (up to 35%), whereas in other Siberian and East Asian populations it was markedly lower (less than 10%). On the contrary, our Y-chromosome data demonstrate that 67% of the South Siberian Y-chromosomes are of Eastern European descent. The Central/Eastern Asian influence has been estimated to be no more than 33%. Overall, the substantial degree of genetic differentiation within South Siberia region was demonstrated using both mtDNA and Y-chromosome haplogroup frequency data. Hierarchical analysis of genetic diversity reveals that geographic distances are the most important factor in genetic differentiation observed.

**An association study between Angiotensinogen (AGT) variants and AGT plasma level indicates functional evolution of the AGT gene.** *L. Fejerman<sup>1</sup>, N. Bouzekri<sup>1</sup>, X. Wu<sup>2</sup>, A. Adeyemo<sup>3</sup>, A. Luke<sup>2</sup>, X. Zhu<sup>2</sup>, R. Ward<sup>1</sup>, R.S. Cooper<sup>2</sup>.* 1) Dept. of Biological Anthropology, Oxford University, Oxford, UK; 2) Dept. of Preventive Medicine, Loyola University Medical Center, Maywood, IL; 3) Dept. of Pediatrics, University College Hospital, University of Ibadan, Ibadan, Nigeria.

During the last ten years, there has been an increasing interest in the associations between different angiotensinogen (AGT) variants and the predisposition to high blood pressure as well as in the relationship between AGT variants and AGT plasma levels. So far, evidence has emerged to support the relationship between AGT variants and the protein level. However, further analysis is needed to define which variants are more directly associated with AGT levels. An evolutionary approach that takes into account the phylogenetic relationship between all the polymorphisms along a given genetic region can enhance our understanding of the genetic nature of quantitative traits. In this study, we sequenced a 6.8 kb region in 57 Nigerian individuals (29 with high AGT plasma levels and 28 with low AGT plasma levels). Haplotypes were determined and grouped in 7 major haplogroups. Their phylogenetic relationship was established and the association of AGT levels and particular haplogroups were investigated. A significant correlation between the genetic distance and AGT levels for the 7 major haplogroups was detected. Our results indicate as plausible that the mutations that occurred during the evolutionary history of the AGT gene leading to the low and high phenotypes, have been accumulating progressively within the two main tree branches in a non-random manner. We propose a hypothesis of the functional evolution of the AGT gene based on this study.

**Geographic and haplotype structure of polymorphisms in *MTHFR*.** *M. Bamshad*<sup>1,2</sup>, *S. Wooding*<sup>2</sup>, *D. Dunn*<sup>2</sup>, *L.B. Jorde*<sup>2</sup>, *R. Weiss*<sup>2</sup>. 1) Dept of Pediatrics, U of Utah, Salt Lake City, UT; 2) Dept of Human Genetics, U of Utah, Salt Lake City, UT.

Polymorphisms in *MTHFR*, the gene encoding the enzyme, 5,10-methylenetetrahydrofolate reductase, influence the metabolism of folate and homocysteine, and are thought, in turn, to modulate the risk of neural tube defects, vascular dementia, and colon cancer. The two most common of these polymorphisms, C677T and A1298C, cause amino acid substitutions that reduce the enzymatic activity of *MTHFR* by ~65% and ~45%, respectively, in persons homozygous for the mutant genotype. As a consequence, these people may have higher folate requirements. The frequencies of C677T and A1298C vary substantially among populations, suggesting that differences in folate levels among environments might have exposed *MTHFR* to varying selective pressures. To investigate the effects of natural selection and population history on patterns of variation in *MTHFR*, we examined DNA sequence variation in 5.5 kb in all 11 exons and flanking regions from 156 chromosomes collected from African, Asian, and European populations. We found 38 single nucleotide substitutions including 5 non-synonymous and 9 synonymous changes in the coding region and 24 in non-coding regions. C677T and A1298C were both more frequent in Europeans. Of a total of 38 haplotypes discovered, C677T was found on two different haplotypes whereas A1298C occurred on 16 different haplotypes. Two haplotype clusters corresponding to haplotypes containing either C677T or A1298C were found. These clusters had different frequencies across Africa, Asia, and Europe. This suggested that natural selection might similarly have influenced both polymorphisms. Patterns of variation in the total population and each continental group did not, however, deviate significantly from expectations under a neutral model or under a model of population expansion.

**Detecting adaptive molecular polymorphism, lessons from the MHC.** *D. Garrigan*<sup>1,2</sup>, *P.W. Hedrick*<sup>2</sup>. 1) Genomic Analysis & Technology , University of Arizona, Tucson, AZ; 2) Department of Biology, Arizona State University, Tempe, AZ.

Predictions of the neutral theory have become the standard by which cases of balancing selection may be inferred. The immunologically important genes of the major histocompatibility complex (MHC) have long served as the canonical example of balancing selection operating in human populations. Human MHC sequence data are synthesized from a wide range of studies and the extraordinary levels of observed polymorphism are used as a benchmark to examine the power of many widely used tests of neutral evolution. These tests are categorized as being capable of detecting selection in the current generation, recent past, and more distant evolutionary past. We find that balancing selection on the human MHC is not detectable in every generation, population, or every evolutionary lineage. This suggests that either selection on the MHC is heterogeneous or that many of the current neutrality tests lack sufficient power to detect selection consistently. We also note an important inference problem that arises when tests reject long-term neutral evolution. We demonstrate that long-term signals of selection, such as the ratio of nonsynonymous to synonymous substitutions, develop quickly, yet persist for exceptionally prolonged periods of microevolutionary time, even in the absence of further selection. A novel, empirical test of selection is proposed that relies only on the observed distribution of neutral allele frequencies throughout the genome. This test bypasses the traditional population genetics assumptions made by many tests based upon the neutral theory.

**Evolution of human apolipoprotein H gene haplotypes and their significance in the production of antiphospholipid antibodies and binding to anionic phospholipid.** *M.I. Kamboh, D.K. Sanghera, H. Mehdi, C.S. Nestlerode, Q. Chen, C.H. Bunker.* Grad Sch Public Hlth, Univ Pittsburgh, Pittsburgh, PA.

Apolipoprotein H (APOH), also known as 2-glycoprotein I is a major autoantigen for the production of antiphospholipid antibodies (APA) in autoimmune diseases. APA is also recognized by a cryptic epitope generated following the interaction of APOH with anionic phospholipids (PL). The prevalence of APA in the general U.S. white population is about 10% but it ranges 30 - 70% in patients with lupus and antiphospholipid syndrome. Since the structural characterization of APOH from different mammalian species is important to identify the evolutionarily conserved regions that may be critical for its function, we have previously determined the chimpanzee APOH gene structure and the prevalence of APA. There is only two amino acid difference between the chimpanzee and human wild type APOH proteins. Chimpanzees have unusually high prevalence (64%) of APA. There is a common protein polymorphism in the human APOH gene with the occurrence of four alleles *APOH\*1*, *APOH\*2*, *APOH\*3* and *APOH\*4*, the latter being present only in blacks. Based on its differential reactivity with an APOH monoclonal antibody, the *APOH\*3* allele is further divided into *APOH\*3<sup>W</sup>* (present only in whites) and *APOH\*3<sup>B</sup>* (present only in blacks). In this study we have screened a large African population (n = 755) to determine the prevalence of APA and the molecular bases of the protein polymorphism. Almost 50% of the Africans were found to be positive for APA. The *APOH\*3<sup>B</sup>* allele was found to be identical to the chimpanzees wild type APOH. Novel two-site or three-site haplotypes, encoded in the third domain of APOH, explained the molecular basis of the *APOH\*3<sup>B</sup>*, *APOH\*3<sup>W</sup>* and *APOH\*4* alleles. Based on the comparison of the human and chimpanzee APOH DNA sequences, we suggest that the *APOH\*3<sup>W</sup>* and *APOH\*4* arose on the ancestral *APOH\*3<sup>B</sup>* haplotype after the split of human races in whites and blacks, respectively. We also found that these haplotypes are associated with the occurrence of APA. Recombinant APOH haplotypes, expressed in COS-1 cells, showed that these mutations also affect the binding of APOH to anionic PL.

**Mitochondrial DNA diversity in Slavonic-speaking populations.** *B. Malyarchuk<sup>1</sup>, T. Grzybowski<sup>2</sup>, M. Derenko<sup>1</sup>, A. Lunkina<sup>1</sup>, G. Denisova<sup>1</sup>, J. Czarny<sup>2</sup>, D. Miscicka-Sliwka<sup>2</sup>.* 1) Genetics Lab, Biological Probl of the North, Magadan, Russia; 2) The Ludwik Rydygier Medical University in Bydgoszcz, Bydgoszcz, Poland.

Maternally inherited mitochondrial DNA (mtDNA) sequence variation was examined by HVS1/HVS2 sequencing combined with SNP-RFLP typing in 885 individuals from European Slavonic-speaking populations inhabiting the territories from the Balkan Peninsula (on the south) to the Baltic Sea coast (on the north) and to the Volga river (on the east). Populations studied included ethnic groups from the West Balkans (Bosnians and Slovenians), Central-North Europe (Poles) and East Europe (Russians). Phylogenetic analysis suggested that maternal lineages were classified into 38 clusters representing the main West Eurasian mtDNA haplogroups. East Eurasian genetic contribution was found at frequency of 1.8% in Poles, 1.6% in Russians and 1.4% in Bosnians. The distribution of mtDNA lineages in Slavonic and the neighboring European populations revealed that the common genetic substratum appears to be characteristic for Central and Eastern European populations (such as Germans, Poles, Russians and Finns). Additionally, according to AMOVA results, South Slavonic populations were found to be significantly different from the neighboring South European populations (Italians, Albanians, and Greeks). It is noteworthy that Slavonic-speaking populations, including the Balkan ones, are characterized by a high frequency (30%-50%) of Y chromosome haplogroup R1a, which is rare in Western Europe. This pattern of mtDNA and Y chromosome distribution is compatible with the archaeological and historical data suggesting that the Slavonic migration waves from Central Europe reached the Balkans, as well as the East European plain, in the early Middle Ages. The work is supported by RFBR grant (03-04-48162).

**SNPSTR autosomal genohaplotypes by restriction site engineering: empirical gametic phase without allele-specific primers.** *A. Knight*<sup>1</sup>, *I.G. Udina*<sup>2</sup>, *B.M. Henn*<sup>1</sup>, *P.A. Underhill*<sup>3</sup>, *J.L. Mountain*<sup>1,3</sup>. 1) Anthropological Sci, Stanford Univ, Stanford, CA; 2) N.I. Vavilov Inst of General Genetics, Russian Academy of Sci, Moscow, Russia; 3) Genetics, Stanford Univ, Stanford, CA.

SNPSTRs have provided, for the first time, a method to simultaneously determine both genotype and phase (the genohaplotype) for compound single nucleotide (SNP) or indel polymorphisms and nearby linked short tandem repeat (STR or microsatellite) loci in autosomes using rapid, multiplex-capable, conventional fluorescent size analysis methods. A drawback to replacement of conventional STR analysis with SNPSTR analysis has been the technical difficulty of optimizing robust allele-specific PCR parameters for the SNP genotyping aspect of the standard SNPSTR approach. Although allele-specific difficulties may be overcome through experimentation, and large-scale population studies are well underway, we endeavored to develop a more reliable and straightforward alternative approach. Here we describe a simple method that entirely circumvents the inherent challenges of allele-specific PCR associated with SNPSTR methodology. This innovative restriction enzyme assay involves use of a forward PCR primer that incorporates a portion of a restriction endonuclease cleavage site (either native or engineered), followed by PCR and digestion, to diagnose the SNP and a fluorescent-labeled reverse primer to indicate STR length by capillary electrophoretic detection on a genetic analyzer. Analysis of SNPSTR genohaplotypes on human chromosome 8 demonstrates the technical ease and utility of the method.

**A method for inferring evolutionary relationships among haplotypes, exemplified by haplotypes at the monoamine oxidase locus.** *H. Nguyen*<sup>1</sup>, *K. Roeder*<sup>1</sup>, *B. Devlin*<sup>2</sup>, *B.S. Maher*<sup>3</sup>, *L.M. Yu*<sup>4</sup>, *R.E. Ferrell*<sup>4</sup>, *M.M.*

*Vanyukov*<sup>5</sup>. 1) Department of Statistics, Carnegie Mellon University, Pittsburgh, PA; 2) Department of Psychiatry, Carnegie Mellon University, Pittsburgh, PA; 3) Div of Oral Biology, University of Pittsburgh School of Dental Medicine, Pittsburgh, PA; 4) Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; 5) Department of Pharmaceutical Sciences, University of Pittsburgh School of Pharmacy, Pittsburgh, PA.

In the search for genetic variants affecting liability to complex diseases, haplotype data have received increasing recognition. When there are more than a few haplotypes, one way to assess the association between disease status and haplotypes is to reconstruct their evolutionary history, i.e. to build a tree that specifies the relative age of each haplotype. To achieve that goal, we combine information from two sources: (1) the inferred network relating haplotypes; and (2) the frequency and mutational history of each haplotype. The former resolves into a space of candidate trees while the latter entails probabilistic modeling to determine the absolute age of each haplotype. To maximize the 'tree likelihood', or the joint likelihood of absolute ages of haplotypes for a given tree, we develop a quasi-boundary procedure that enables us to compare trees consistent with the inferred network. These comparisons make explicit which trees are most likely to have generated the observed data and thus guide decisions for data analysis. We evaluate these methods using data on monoamine oxidase (MAO) haplotypes from two ethnic backgrounds, African-American and European-American. Our results show that the evolutionary trees exhibit consistency of relative ages among common haplotypes in the two ethnic groups.

**Progress toward the identification of origins of myotonic dystrophy type 1 mutation: worldwide study in the CEPH-Human Genome Diversity Project.** *T. Miki<sup>1</sup>, H. Yamagata<sup>1,2</sup>, S. Akiyama<sup>3</sup>, Y. Chen<sup>1</sup>, K. Kohara<sup>1</sup>, I. Kondo<sup>2</sup>.*

1) Dept Geriatric Medicine, Ehime Univ Sch Medicine, Ehime, Japan; 2) Dept Med Genetics, Ehime Univ Sch Medicine, Ehime, Japan; 3) Dept Clin Nursing, Sch Health Sciences, Univ Occupational and Environmental Health, Fukuoka, Japan.

Background: Although it is believed that a major ancient mutation underlying myotonic dystrophy type 1 (DM1) has originated from reservoir pool (CTG19-37), the molecular mechanisms underlying repeat instability are not well understood. Objectives: To understand the origins of DM1 mutations and the evolution of modern humans. Samples and Methods: 1064 normal individuals from 51 different world populations in 21 countries (CEPH-HGDP) and Japanese DM1 samples were analysed. We determined CTG-repeat number and examined 8 SNPs at the 20kb DMPK-SIX5 region. Determination of genotypes included GeneScan (CTG repeat) and PCR-RFLPs (SNPs). Linkage disequilibrium and haplotype analysis were carried out. Results and Discussion: As for CTG repeat distributions, the results were similar to those reported by Tishkoff et al.(1998). Some populations showed trimodal distribution (CTG5, 6-17, 18-37) and others did not. Frequencies of CTG5, 11-13, >19 were grouped. As expected, DM1 rare populations (sub-Saharan African, China etc) had no CTG>19 repeats. As for Amerindians (Karitiana, Maya etc), a major genetic bottleneck from Asia to the Americas was seen. The SNPs in promoter region of DMPK showed an evenly distributed pattern when normal alleles were grouped according to CTG repeat length. Even for CTG>20 alleles there were different haplotypes according to SIX5 locus SNPs. It suggests that CTG>20 alleles are also comparatively stable and not all >20 repeat allele will increase its size in successive generations. Common DM1 founder chromosome region was postulated to be within about 17kb including CTG repeat. Our data suggest founder chromosomes rather than predisposing alleles.

**Integration of association statistics over genomic regions using BARS.** *K. Roeder<sup>1</sup>, X. Zhang<sup>2</sup>, D. Wallstrom<sup>1</sup>, B. Devlin<sup>3</sup>.* 1) Dept Statistics, Carnegie Mellon Univ, Pittsburgh, PA; 2) Dept of Biometrics Research Merck Research Laboratories; 3) Dept of Psychiatry, University of Pittsburgh School of Medicine.

In the search for genetic determinants of complex disease, two approaches to association analysis are most often employed, testing single loci or testing a small group of loci jointly via haplotypes for association to disease status. It is still debatable which of these approaches is more favorable, and under what conditions. The former has the advantage of simplicity but suffers severely when alleles at the tested loci are not in linkage disequilibrium (LD) with liability alleles; the latter should capture more of the signal encoded in LD, but is far from simple. The complexity of haplotype analysis could be especially troublesome for association scans over large genomic regions, which in fact is becoming the standard design. For these reasons, we have been evaluating statistical methods that bridge the gap between single-locus and haplotype-based tests. In this article we present one such method, which uses non-parametric regression techniques embodied by Bayesian Adaptive Regression Splines (BARS). For a set of markers falling within a common genomic region and a corresponding set of single locus association statistics, the BARS procedure integrates these results into a single test by examining the class of smooth curves consistent with the data. The non-parametric BARS procedure generally finds no signal when no liability allele exists in the tested region (i.e., it achieves the specified size of the test) and yet it is sensitive enough to pick up signals when an liability allele is present. The BARS procedure provides a robust and potentially powerful alternative to classical tests of association, diminishes the multiple testing problem inherent in those tests, and can be applied to a wide range of data types, including genotype frequencies estimated from pooled samples.

Program Nr: 1206 from 2003 ASHG Annual Meeting

**Mitochondrial haplotypes revealed genetic connection between Sino-Tibetan and Japanese.** *Y. Qian, L. Jin, B. Su.*  
Center for Genome Information, Dept. Env. Health, Univ Cincinnati, Cincinnati, OH.

To investigate genetic affinity among East Asian populations, a total of 515 mtDNA lineages of 14 Eastern Asian populations, including 6 Sino-Tibetan speaking populations, 2 Japanese populations from East and West Honshu respectively, and 2 Altaic, 1 Daic and 3 Astro-Asiatic speaking populations were involved in this study. Ten Eastern Asian-specific RFLP markers in coding region were typed and non-coding region HVS-I (16042-16569 following 1-41) were sequenced to determine haplotypes of each mitochondrial lineage. Based on published data and our dataset, a phylogenetic tree of haplogroups was generated and allowed us to describe matrilineal relationships of these populations. Our results showed that all mtDNA lineages are affiliated with two macrohaplogroup M and N, however, the distribution of sub-haplogroup frequency showed differences between Daic, Astro-Asiatic populations which are genetically close to Southeast Asian populations and Sino-Tibetan, Altaic, Japanese populations. By contrast, Japanese exhibited similar haplogroup frequency distribution with Sino-Tibetan and Altaic populations, especially for a few geographic specific haplogroups, such as F1b, D, G1 and G2a, indicating close genetic affinity with Northern Chinese populations. Principal components analysis also suggested such genetic connection between Japanese and these Chinese populations. Interestingly, the mitochondrial data indeed support our previous observation that inferred migration from Himalayas to Japan using Y chromosome polymorphic markers typing.

**Haplotype analysis of the M390R mutation within *BBS1*, the most common locus for Bardet-Biedl syndrome.** A.J. Ross, B.E. Hoskins, P.J. Scambler, P.L. Beales. Molecular Medicine Unit, Institute of Child Health, London, United Kingdom.

Bardet-Biedl syndrome (BBS) is a heterogeneous disorder with primary features that include age-related retinal dystrophy, obesity, polydactyly, renal dysplasia, reproductive tract anomalies and cognitive impairment. It is genetically heterogeneous and has at least seven loci, with mutations identified in five genes to date (*BBS1*, *BBS2*, *BBS4*, *BBS6* & *BBS7*). The *BBS1* gene is the locus most frequently involved with this disorder, with mutant alleles present in 20% of all patients. M390R, a missense mutation within exon 12 of *BBS1*, is the most common BBS mutation and has been shown to be involved in 18-32% of all BBS cases. This single mutation, which accounts for the vast majority (75%) of all *BBS1* associated alleles, is found almost exclusively in patients of European origin. Therefore, it has been suggested that M390R may be an ancient mutation that has arisen from a single common ancestral event, which has subsequently been fixed within this population. In support of this, results from a recent study typing STRP markers from an approximately 450Kb region flanking the *BBS1* gene in patients from 22 families have indicated that there may be a common M390R haplotype. Here, we present the results of haplotype analyses of BBS patients with the M390R mutation from 24 unrelated families of European origin using the same STRP markers to facilitate direct comparison. Our results provide further evidence to suggest that M390R is likely to be an ancient mutation, which has possibly been maintained in the European population through positive M390R heterozygous advantage.

Program Nr: 1208 from 2003 ASHG Annual Meeting

**The use of SNP-based haplotypes and LD blocks to positionally clone modifying determinants of expansion of the Fragile X triplet repeat.** *S. Ennis, A. Collins, N.E. Morton.* Human Genetics Division, Duthie Building (MP808), Southampton General Hosp, Southampton, United Kingdom.

Unstable germline transmission of the FRAXA CGG triplet repeat at Xq27.3 results in transcriptional silencing of the adjacent FMR1 gene when the repeat tract exceeds 200 units. The resulting Fragile X phenotype is the most common form of inherited mental retardation affecting ~1/5500 males. Purity and length of the CGG array influence stability but other cis-acting factors are postulated to exist. Over the last decade, three microsatellite repeats (DXS548, FRAXAC1 and FRAXAC2), have been commonly applied to characterise the haplotypic background upon which a FRAXA allele occurs. Although high-risk haplotypes have been identified using these markers, causal determinants of expansion remain elusive.

We have typed a panel of 22 single nucleotide polymorphisms (SNPs) across 650 kb of the FRAX region on a panel of 877 independent male chromosomes that span the range of FRAXA repeat sizes. The resultant haplotypes confirm and extend the information gleaned from microsatellite haplotypes. A metric linkage disequilibrium map of the region was created to identify discrete regions of elevated recombination and extended regions containing contiguous SNPs in high LD.

These SNP data were then used in a program recently developed for the ALLASS suite of software, which reads data from SNP haplotypes, and uses linkage disequilibrium to positionally clone disease genes. As the causal mutation for fragile X syndrome is already known, the program was instead used to examine the regional marker data for: 1) evidence for and; 2) the location of a modifier of dynamic mutation. Results indicate a region 3 of the FMR1 gene is significantly associated with expanded CGG repeats. Further examination of this associated region using SNPs at increased density may reveal some genetic component of the FMR1 CGG repeat expansion.

**MJD haplotypes unequally distributed throughout mainland Portugal.** *S. Martins*<sup>1,2</sup>, *C. Gaspar*<sup>3</sup>, *I. Silveira*<sup>2</sup>, *F. Calafell*<sup>4</sup>, *G. Rouleau*<sup>3</sup>, *P. Coutinho*<sup>5</sup>, *A. Amorim*<sup>1,6</sup>, *J. Sequeiros*<sup>2,7</sup>. 1) IPATIMUP, Portugal; 2) UnIGENe, IBMC, Univ Porto, Portugal; 3) Centre for Research in Neuroscience, McGill Univ, Montreal General Hosp, Canada; 4) Unitat de Biologia Evolutiva, Fac Ciències Salut Vida, Univ Pompeu Fabra, Spain; 5) Serv Neurologia, Hosp S Sebastião, St<sup>a</sup> M<sup>a</sup> Feira, Portugal; 6) Fac Ciências, Univ Porto, Portugal; 7) ICBAS, Univ Porto, Portugal.

Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disorder, caused by an unstable CAG expansion in the *MJD1* gene. The disease was first described in families of Portuguese-Azorean ancestry, but the origin of the mutation(s) has been a matter of debate since it was suggested its introduction in northeastern Portugal, spreading later to the Azores archipelago, and from there to the rest of the world. More recently, a haplotype study, using three intragenic SNPs, showed the association of an A-C-A and a G-G-C haplotypes with MJD in Azorean families, respectively from the islands of Flores and S. Miguel. Worldwide, the majority of the families shared the A-C-A haplotype, suggesting a major founder mutation. In order to clarify the origin and spread of the MJD mutation(s), we have (1) performed a haplotype analysis, including the CAG polymorphism and the intragenic SNPs A<sup>669</sup>TG/G<sup>669</sup>TG, C<sup>987</sup>GG/G<sup>987</sup>GG, TAA<sup>1118</sup>/TAC<sup>1118</sup> and a newly identified one, in MJD mainland Portuguese families, and (2) searched for microsatellites flanking the CAG repeat. The new intragenic polymorphism is located in intron 7 (IVS7-86TG). Strong linkage disequilibrium was noted between T and G alleles, from IVS7-86TG and A<sup>669</sup>TG/G<sup>669</sup>TG, respectively. The preliminary haplotype analysis showed a heterogeneous distribution over mainland Portugal. Interestingly, the most frequent haplotype was G-G-C (in contrast to the rest of the world), except in northeastern Portugal, where A-C-A seems to be over-represented. In addition, we found a polymorphic GT repeat, 227 kb from the CAG, with five alleles in the MJD population. By sequencing four of them, we have identified three different lineages caused by nucleotide substitutions. These results are compared with those in a large control population.

Program Nr: 1210 from 2003 ASHG Annual Meeting

**Evidence for epistasis between chromosome 2p and 13q in human obesity.** *C. Dong, W.D. Li, D. Li, R.A. Price.*  
Dept Psychiatry, Univ Pennsylvania, Philadelphia, PA.

We employed several model-free approaches to conduct multipoint analyses and look for epistatic interactions in human obesity using our second-generation genome scan data in 1297 individuals from 260 European American families. For assessment of epistasis, we found the strongest evidence between chromosomes 2p24-p25 and 13q12-q14 with a correlation score of 0.213 (N=247, p=0.0008) for BMI 35 and 0.406 (N=115, p=6.6-E6) for BMI 40 based on alleles shared identical by descent (IBD) for affected sib pairs (ASP) and with a correlation score of 0.306 (N=201, p=1.0-E5), 0.339 (N=161, p=1.1-E5) and 0.426 (N=98, p=1.2-E5) for BMI 30, 35 and 40, respectively, based on family-specific nonparametric linkage (NPL) scores. We also conducted NPL score-based conditional logistic regression analyses and found a significant interaction between the two regions with a regression coefficient for the interaction term of 0.706 (p=0.0002), 0.810 (p=0.0002) and 1.258 (p=0.0001) for BMI 30, 35 and 40, respectively. We performed conditional analyses using the ALLEGRO program and found that the LOD score at 2p rises from 0.06 in the baseline analyses to 2.00, 2.36 and 1.96 for BMI 30, 35 and 40, respectively, when families were weighted by evidence for linkage at 13q (26 cM) using zero-one weights. For a continuous BMI phenotype, we conducted Haseman-Elston regression analysis based on a two-locus epistatic model, which yielded significant evidence for interaction between 2p (28 cM) and 13q (LOD=4.11), compared with a two-locus model (LOD=1.20). Results from variance component analysis also supported the observed interaction between the two regions by showing an epistatic effect of p=0.004. Our findings suggest that genetic loci on chromosomes 2p and 13q may interact to influence human obesity. (This study was supported in part by NIH grants R01DK44073, R01DK48095, and R01DK56210 to RAP.).

**Combined and sex-specific linkage analyses of thyroid hormones in Mexican Americans.** *N. Gouin<sup>1</sup>, M.M. Barmada<sup>2</sup>, G. Perez<sup>2</sup>, C. Kammerer<sup>2</sup>, S. Cole<sup>1</sup>, H. Göring<sup>1</sup>, J. Blangero<sup>1</sup>, L. Almasy<sup>1</sup>, D. Finegold<sup>3</sup>, T.P. Foley<sup>3</sup>, P. Samollow<sup>1</sup>.* 1) Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 3) Department of Endocrinology, UPMC Health System, Pittsburgh, PA.

Thyroid hormones play major roles in the regulation of protein synthesis, basal metabolism, and lipid metabolism. In an effort to understand the genetic regulation of thyroid hormone levels, we performed variance components linkage analyses to detect quantitative trait loci (QTLs) using data on six thyroid hormone or thyroid hormone related traits (free and total thyroxine (FT<sub>4</sub> & TT<sub>4</sub>), free and total triiodothyronine (FT<sub>3</sub> & TT<sub>3</sub>), thyroxine-binding globulin (TBG), thyrotropin (TSH), and on insulin-like growth factor 1 (IGF1). Phenotypic and genotypic data were available on 1022 individuals (428 male and 595 females) from 33 Mexican-American families collected as part of the San Antonio Family Heart Study. Prior to performing linkage analyses over both sexes and within male and female subgroups, significant effects of measured external environmental components were identified via multivariate linear regression. Overall, we detected suggestive evidence for QTLs influencing TT<sub>3</sub> on chr 16q (LOD=2.78), FT<sub>4</sub> on chr 11p (LOD=2.43), and IGF1 on chr 8 (LOD=2.57). Sex-specific analyses indicated that evidence for the overall IGF1 QTL was mostly from the subgroup of women, whereas each sub-group contributed to evidence for the TT<sub>3</sub> and FT<sub>4</sub> QTLs. We also detected a QTL for FT<sub>4</sub> levels on chr 16q (LOD=3.14) in women, which was not evident in men or the combined group; this QTL is within 20cM of the TT<sub>3</sub> QTL. Our results illustrate that analyses performed overall and within subgroups will enhance our understanding of genetic control of thyroid hormone variation and also facilitate the identification of possible QTLs that influence thyroid hormone function.

**Results from web-based recruitment of subjects for study on the genetics of absolute pitch.** *B. Levinson<sup>1</sup>, J. Zemansky<sup>1</sup>, A. Athos<sup>1</sup>, A. Kistler<sup>1</sup>, N. Freimer<sup>2</sup>, J. Gitschier<sup>1</sup>*. 1) Dept. of Medicine and Pediatrics, University of California, San Francisco and HHMI; 2) Center for Neurobehavioral Genetics, Neuropsychiatric Institute, and Dept. of Human Genetics, University of California, Los Angeles.

Absolute pitch (AP), also known as perfect pitch, is the ability to identify the pitch of a tone without use of a reference. Previous studies by our group and others have shown evidence for both a genetic and environmental contribution to the development of AP. To map the gene(s) responsible for AP, we are collecting affected relative pairs and trios. For efficient recruitment of subjects, we rely on a web-based survey and pitch-identification test (<http://perfectpitch.ucsf.edu>). From 7/31/02 to 6/17/03, 764 people completed the survey and took the acoustical test, which includes both pure tones and piano tones. Of these, 316 scored sufficiently high in pitch-recognition (AP-1) to be included in our study. Subjects were accurate in their self-description, with 80% of subjects who indicated AP ability testing AP-1, and only 11% of those answering no or don't know testing AP-1. Age range for subjects entering the study is 6 to 79, with 60% falling between ages 14 and 30, a skewed distribution most likely reflecting increased use of the web in this age group. Subjects testing AP-1 ranged from 12 to 67 with a similarly skewed distribution. 28% of responding AP-1 subjects report a relative with AP ability (more than 50% indicated a sibling), with an additional 34% unsure. AP-1 subjects also proved to be quite accurate in their assessment of their family members' pitch abilities, as only a handful of reported relatives failed to test AP-1. Confirmed families were invited to contribute a blood sample, collected by a mobile phlebotomy service. Samples have been immortalized and stored. Our large data set also allows us to analyze the errors in AP-1 subjects tests. Subjects tend to err sharp more than flat. Of interest was a common error in perception in multiple octaves of the pure tone G# as an A. This error was not found in the piano tone test. Errors were also more frequent in older participants.

**The Role of CARD15/NOD2 variants G908R, R702W and L1007fsins in a pediatric Crohn's Disease cohort.** *K.L. Maresso<sup>1</sup>, N. Collins<sup>2</sup>, R. Kraemer<sup>1</sup>, S. Kugathasan<sup>2</sup>, U. Broeckel<sup>1</sup>.* 1) Human & Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI; 2) Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI.

Three single nucleotide polymorphisms (SNPs) within the CARD15/NOD2 (G908R, R702W, L1007fsins) gene have been strongly associated with susceptibility to Crohn's Disease (CD) in various adult populations. While there are distinct clinical patterns between adult- and pediatric-onset CD, no study has yet to examine the role of these SNPs in childhood CD using a population-based, pediatric cohort. Consequently, we aimed to determine allele frequencies of G908R, R702W and L1007fsins within children and to test for their linkage and association with pediatric-onset CD. Determining the role of these mutations within pediatric CD will assist in identifying molecular genetic differences between adult and childhood forms of the disease. A total of 159 patients have been recruited from a population-based, pediatric CD registry in southeastern Wisconsin. We have parental DNA for 40% of these patients, constituting 63 complete mother-father-child trios. All patient and parental DNA samples were genotyped for the three SNPs using direct fluorescence-based sequencing. Allele frequencies were calculated for all patients and the Transmission-Disequilibrium-Test (TDT) was used to assess linkage and association within the trios (GeneHunter, v.2.0). Allele frequencies within the affected children were as follows: G908R, 5.2%; R702W, 7.6%; and L1007fsins, 10.1%. These frequencies are similar to those found in adult populations. Results of both the single-locus ( $\chi^2 = 7.36$ ,  $p = 0.007$ ) and two-locus ( $\chi^2 = 4.5$ ,  $p = 0.03$ ) TDT demonstrated that the L1007fsins allele is preferentially transmitted from parent to affected offspring. Based on this preliminary analysis, the L1007fsins appears to play a role in pediatric CD. However, it is likely there are additional genetic determinants influencing susceptibility to pediatric CD, as allele frequencies within this population are similar to those of adult CD populations. This is the first such study of Crohn's Disease-associated polymorphisms in a population-based, pediatric cohort.

Program Nr: 1214 from 2003 ASHG Annual Meeting

**Worldwide genetic variation in human bitter taste receptor genes.** *U.-K. Kim, J. Larsen, D. Drayna.* NIDCD/NIH, Rockville, MD.

Common allelic variants of a member of the TAS2R bitter taste receptor gene family underlie variation in the ability to taste phenylthiocarbamide (PTC). To extend these results to other bitter receptors, we have sequenced 22 of the 24 known TAS2R genes in a series of populations worldwide, including Hungarians, Japanese, Cameroonians, Pygmies, and South American Indians. All 22 TAS2R genes contain common SNPs within their coding sequence, and we identified an average of 4.4 SNPs per TAS2R gene. Fifteen variants listed in dbSNP were not observed to be polymorphic in our sample. 77% of the SNPs we observed cause an amino acid substitution in the encoded receptor protein, giving rise to a very high degree of receptor protein variation in the population. Four SNPs specify one allele that introduces an in-frame stop codon in the gene. The frequency of these stop codon alleles indicates that there are a significant number of individuals who are homozygous null for some bitter taste receptors. Overall, African samples displayed higher diversity of alleles. This is consistent with the view that the majority of human genetic variation resides within older African populations, and a fraction of this variation emerged and subsequently spread across the remainder of the world.

**Linkage disequilibrium and haplotype structure of four SNPs of the Interleukin-1 gene cluster in seven South Asian populations.** *S.M. Raju<sup>1</sup>, R. Govindaraju<sup>1</sup>, N. Wang<sup>2</sup>, R. Chakraborty<sup>2</sup>.* 1) 4 Marshall Road, Lexington, MA; 2) Center for Genome Information, Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, OH.

We investigated the extent and pattern of genetic variations at four single nucleotide polymorphism sites (SNPs: IL-1A 4845, IL-1B 3954, IL-1B 511, and IL1-RA 2018) of the interleukin-1 gene cluster among 280 unrelated individuals, representing seven caste groups (Bunt, Havyak, Iyenger, Lingayath, Smartha, Vaishya, and Vokkaliga) from the state of Karnataka, India, and one European American community of Boston area, US. In terms of allele frequencies at these sites, two- to four-fold differences were found among these eight populations. Genotype frequencies at these sites were, by and large, in Hardy-Weinberg equilibrium in all populations. Linkage disequilibrium (LD), examined by  $D$ ,  $R^2$ , and Fishers exact test, showed significant allelic associations between sites, but the strength of LD varied by site-pairs as well as across populations. Haplotype frequency distributions and haplotype diversity, inferred by the E-M algorithm, also showed considerable differences across populations, some of which were statistically significant. The frequency of the most common haplotype ranged from 15 to 40 percent. Presence of more than 5 segregating haplotypes (e.g., 7 in the Bunt, and 11 in the Caucasian sample) in multiple populations provides evidence of historical recombinations between sites. Together, these observations indicate a substantial inter-population variation of the extent and pattern of polymorphisms of the interleukin-1 genes, suggesting that these polymorphisms may differentially influence susceptibility to autoimmune diseases among the studied populations. Differences of community sizes, and traditions of consanguinity may account for the observed population differentiation within small geographic areas at these functionally relevant SNP sites. These observations also imply that factors responsible for such population differentiation may impact inference regarding haploblock structures in specific genomic regions. (Research supported by US Public Health Service Research grants to RC).

**Ipf-1 D76N mutation is present in Italian late-onset type 2 diabetics.** *J.F. Brittman<sup>1</sup>, J.F. Habener<sup>1,2,3</sup>, C. Gragnoli<sup>1,2,4</sup>*. 1) Molecular Endocrinology, MGH, Boston, MA; 2) Med. School., Harvard. Univ., Boston, MA; 3) HHMI, Boston, MA; 4) Univ. Tor Vergata of Rome, Italy.

Maturity onset diabetes of the young (MODY) is a monogenic autosomal-dominant subform of type 2 diabetes mellitus (T2DM). It has an onset before age 25 and is due to mutations in at least six different genes. MODY gene mutations are also a cause of late-onset T2DM. Genetic variations in insulin promoter factor-1 (Ipf-1/MODY4) are uncommon but when present contribute to diabetes as part of a polygenic background. IPF-1 is a homeodomain transcription factor required for pancreas development and transcriptional regulation of pancreatic beta cell genes. Our goal was to investigate whether ipf-1 mutations are responsible for late-onset T2DM in the Italian population. We recruited and screened 89 late-onset T2DM Italian probands for Ipf-1 mutations in exon 1-2 and flanking introns. We identified one subject with a single nucleotide substitution GAC to AAC at AA Asp76. 50 Italian controls with negative family history for T2DM and normal fasting glucose were directly sequenced for the presence of Ipf-1 Asp76Asn. We excluded variations in exon 1 in the 100 normal chromosomes. The affected T2DM sib of the mutant proband is also a D76N mutation carrier. This mutation D76N has been already identified in the French, UK, Swedish and Danish late-onset T2DM population and it is known to be located in the transactivation domain of IPF-1 and to affect the IPF-1 transactivation and DNA-binding activities. Furthermore, the AA 76 is highly conserved in hamster, rat and mouse. This is the first screening of Ipf-1 gene performed in the late-onset T2DM Italian population, while a previous screening in an early-onset T2DM Italian population identified a P33T mutation. So far in multiple population screenings of the Ipf-1 gene, 12 different mutations have been identified. D76N Ipf-1 has a prevalence of 1.1 per cent in the late-onset T2DM Italian population and represents the most common mutation present in Ipf-1, and contributes to late-onset T2DM with a prevalence of 1.9 per cent in the European population (combined estimate from published data and this described study).

**Gene diversity of mitochondrial DNA in Russians.** *O.P. Balanovsky*<sup>1, 2</sup>, *R. Villems*<sup>2</sup>, *M.I. Churnosov*<sup>3</sup>, *E.A. Pocheshkova*<sup>4</sup>, *E.V. Balanovska*<sup>1</sup>. 1) Research Centre for Medical Genetics, Moscow, Russia; 2) Estonian Biocenter, Tartu, Estonia; 3) Belgorod State University, Belgorod, Russia; 4) Kuban Medical Academy, Maikop, Russia.

We have analyzed mtDNA variability in 640 Russians to evaluate degree and patterns of intra-ethnic variability of mitochondrial genome. The studied sub-populations are: northern (from Arkhangelsk region), western (Smolensk region), eastern (Kostroma region), southern (Belgorod region) and the Kuban Cossacs, with a median distance between the groups more than 1000 km. The first hypervariable segment was sequenced in all samples and a set of informative RFLP sites was typed. We have found that the other East Slavonic people Ukrainians and Belorussians - have the largest number of maternal lineages, shared with Russians, whereas western and southern Slavs differ in their haplotype composition from Russians more. Finnic ethnoses, both the Baltic and Volga-Finnic, encompass somewhat more lineages, shared with Russians, than western and southern Slavs do. This can be interpreted in terms of a significant Finno-Ugric substratum in Russians. Multidimensional scaling (MDS) analysis revealed two clusters - Finnic and Slavic, with Baltic language speakers as intermediate. The MDS plot shows that differentiation between maternal lineages of Finnic-speaking ethnoses is greater than that between Slavic populations. However, among the Slavic-speaking people, intra-Russian variability of mtDNA is as high as that among all Slavic ethnoses studied here: northern and southern Russians occupy two poles of the intra-Slavic variation. The northern Russians are the most distant because of specific features of their mtDNA haplogroup composition. We conclude that large geographic distances between Russian sub-populations have resulted in a high level of intra-Russian mtDNA variability that is comparable with inter-ethnic differences in East Europe in general. Several substratum populations, including Finnic-speaking tribes, have likely facilitated the creation of a diversity that can be observed in the extant Russian-speaking people of the East Europe. The work was supported by RFBR (grants 01-06-80085, 01-07-90041).

**Molecular genetic analysis of suballeles of the ABO blood group system in a Kuwaiti population using multiplex PCR-RFLP.** *M.M. El-Zawahri<sup>1</sup>, Y.A. Luqmani<sup>2</sup>, G.C. Abraham<sup>2</sup>, A.A. Al-Bashir<sup>3</sup>*. 1) Dept Biological Sci, Fac Sci, Kuwait Univ, Kuwait; 2) Dept Pharmaceutical Chemistry, Fac Pharmacology, Kuwait Univ, Kuwait; 3) Kuwait Central Blood Bank (KCBB), Kuwait.

The ABO blood group system is one of the conventional polymorphisms that are important in transfusion medicine, forensic serology and anthropological genetics. The Kuwaiti population is a relatively young that has roots originating mainly from Saudi Arabia, Iraq, and Iran. The present study is the first demonstration of an extensive variability linked to suballeles of the ABO locus in a Kuwaiti population. Molecular genetic analysis of suballeles of the ABO blood group system and their distribution were studied by multiplex PCR-RFLP method in 355 unrelated Kuwaiti individuals. DNA was extracted from EDTA blood samples. With multiplex PCR, fragments of 252 (251 for O<sup>1</sup>) and 843 (842 for A<sup>2</sup>) bp were amplified in the same tube using two pairs of specific primers. The amplified fragments cover the two major exons 6 and 7, and constitute 91% of the translated gene. After simultaneous digestion with restriction endonucleases HpaII and KpnI, a conclusive interpretation of the expected fragment pattern obtained could be made in all cases. Fifteen different genotypes could be identified when combining the A<sup>1</sup>, A<sup>2</sup>, B, O<sup>1</sup> and O<sup>2</sup> suballeles from the digestion patterns. The serologic phenotypes of the 355 blood samples used in this study were: A (77), B (97), AB (10) and O (171). The genotypes determined were 1 A<sup>1</sup>A<sup>1</sup> (0.28%), 6 A<sup>1</sup>A<sup>2</sup> (1.69%), 38 A<sup>1</sup>O<sup>1</sup> (10.71%), 1 A<sup>1</sup>O<sup>2</sup> (0.28%), 1 A<sup>2</sup>A<sup>2</sup> (0.28%), 30 A<sup>2</sup>O<sup>1</sup> (8.45%), 6 A<sup>1</sup>B (1.69%), 4 A<sup>2</sup>B (1.13%), 12 BB (3.38%), 79 BO<sup>1</sup> (22.25%), 6 BO<sup>2</sup> (1.69%), 167 O<sup>1</sup>O<sup>1</sup> (47.04%) and 4 O<sup>1</sup>O<sup>2</sup> (1.13%). Two of the combinations (A<sup>2</sup>O<sup>2</sup>, O<sup>2</sup>O<sup>2</sup>) were not found amongst the 355 samples tested. All genotypes determined were consistent with the observed phenotypes. These frequency data may provide useful additional information for forensic medicine, organ transplantation and phylogenetics in Kuwaiti population. Supported by grant SZ 01/00 from Kuwait University.

**Y chromosome haplotype diversity in insular and mainland populations from Croatia.** *L. Barac<sup>1</sup>, M. Pericic<sup>1</sup>, I. Martinovic Klaric<sup>1</sup>, S. Rootsi<sup>2</sup>, B. Janicijevic<sup>1</sup>, T. Kivisild<sup>2</sup>, J. Parik<sup>2</sup>, P. Rudan<sup>1</sup>.* 1) Institute for Anthropological Research, Zagreb, Croatia; 2) Department of Evolutionary Biology, Institute for Molecular and Cell Biology, University of Tartu, Tartu, Estonia.

Y chromosome haplotypes have become invaluable tools for determination of parentage and kinship in forensic casework and population genetics. Eight short tandem repeat (STR) polymorphic systems mapped on the human Y chromosome (DYS19, DYS388, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393) were typed in mainland (n = 109) and four insular (n = 348) populations. A general allelic frequency distribution in the overall Croatian sample corresponds to other European populations with the exception of the loci DYS19 and DYS389II. The most frequent DYS19 allele was 16, while at the DYS389II the most frequent were alleles 30 and 31. Complete typing was obtained for 457 chromosomes that showed two hundred forty one different Y chromosome haplotypes. The most frequent Y chromosome haplotype (16-13-13-31-24-11-11-13) was found in 33 individuals (7.22%). One hundred seventy four haplotypes (38.07%) were observed in single copies. Comparison of insular and mainland populations showed a statistically significant difference in allele frequencies at 4 out of 8 loci. Moreover, the haplotype 16-13-13-32-24-11-11-13 was noticed exclusively in two islands. The haplotype diversity ranged from 0.9749 (island of Korcula) to 0.9913 (mainland). Observed differences between mainland and insular populations could be explained by specific population histories, unlike settling patterns involving different gene boundaries and more pronounced effects of genetic drift in island.

**Cytochrome P450 2D6\*2, \*3, and \*4 polymorphisms in a Mexican population.** *J.L. Guerrero<sup>1</sup>, M. Lopez<sup>2</sup>, G. Galicia<sup>1,2</sup>, E. Alonso<sup>1</sup>.* 1) Genetics and Molecular Biology, Nac. Inst. Neurol. Neuroc., Mexico City, Mexico City, Mexico; 2) Biologic Systems, UAM-X, Mexico, D.F.

Cytochrome P450 2D6 (CYP2D6) catalyzes the oxidative metabolism of several clinically important classes of drugs. The CYP2D6 gene is highly polymorphic, and more than 70 different alleles have been identified. These genetic variants have been correlated to 3 classes of phenotypes based on the extent of drug metabolism: ultra-extensive (UEM), extensive (EM) and poor (PM) metabolizers resulting in low, normal and high blood levels of parent drugs. In addition to individual variation in drug response, there are interethnic differences. Among Caucasians, 5-9% lack functional CYP2D6 enzymes, whereas the incidence of PMs among Asians and Africans is <2%. Molecular analysis of the CYP2D6 locus has also revealed interethnic differences in allele frequencies. The most common mutations in Caucasians are CYP2D6\*4 (G1934A) and CYP2D6\*3 (A2637 del), these alleles are rarely found among Chinese, Koreans, black Americans and black Zimbabweans. The CYP2D6\*2 allele is the most common allele encoding intermediate enzyme activity in Caucasians, and results have a strong effect on the metabolic phenotype when present in combination with a null allele. We studied 152 unrelated healthy Mexican volunteers, between 18 and 55 years old. The leukocyte DNA was isolated and CYP2D6\*1, \*2, \*3 and \*4 sequences were amplified by PCR, followed by digestion with Cfo I, Msp I and Mva I, respectively. DNA fragments were analyzed by electrophoresis. The allelic frequency found was 63.4%, 24.8%, 11.0% and 0.8% for CYP2D6\*1, CYP2D6\*2, CYP2D6\*4, and CYP2D6\*3, respectively. We identified one subject who was homozygous for the CYP2D6\*4 allele, and another individual who had a combination of CYP2D6\*3 and CYP2D6\*4 alleles. The population frequency of the CYP2D6 poor metabolizer phenotype was estimated to be 2%. This study will extend the literature attesting to ethnic differences with respect to CYP2D6 polymorphism. The polymorphic CYP2D6 enzyme activity in Mexican population could have important implications for the use of drugs that are substrates for CYP2D6 and have a narrow therapeutic window. CONACYT, 37103 M.

**Y chromosome and mitochondrial DNA variation and the origin of Lithuanians.** *V. Kucinskas*<sup>1</sup>, *D. Kasperaviciute*<sup>1</sup>, *D. Ambrasiene*<sup>1</sup>, *M. Stoneking*<sup>2</sup>. 1) Dept. of Human and Medical Genetics, Vilnius Univ, Vilnius, Lithuania; 2) Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany.

There are conflicting anthropological findings regarding the origins of Indo-Europeans and formation of Baltic tribes in Eastern Baltics. The Lithuanians and the Latvians are the only Baltic cultures that survived until today, therefore molecular genetic characterisation of these populations may help to reconstruct the prehistory and ethnogenesis of the people in this area. We have analysed mitochondrial DNA and Y chromosome diversity in six ethnolinguistic groups of Lithuanians. Analysis of molecular variance shows that all variation in Lithuania is due to variation within groups, and no variation was detected among groups neither in mtDNA nor Y-chromosome markers. Comparisons of mtDNA sequences with other European populations have not revealed significant differences, however, more lineages are shared with populations of northern Europe. Y-chromosome analysis shows Lithuanian population to be closest to Latvians and Finno-Ugric speaking Estonians, all three populations being indistinguishable in frequencies of biallelic markers. Interestingly, Lithuanians have a high frequency (~37%) of TatC allele, which is frequent in Uralic speakers of northern Eurasia. However, the analysis of Y chromosome 9 different microsatellite markers on TatC allele background in Lithuanians and Estonians revealed significant differences, thus indicating different histories of these populations. Lower gene diversity and nearly star-like median joining network of Lithuanian TatC chromosomes indicates a recent population bottleneck, however major Lithuanian haplotypes are rare in Estonians, suggesting that these populations have different origins or have differentiated before Indo-Europeanization took place in Eastern Baltics.

**Microsatellite genetic variation in the Indian Subcontinent.** *S.S. Mastana*<sup>1</sup>, *G. Sun*<sup>2</sup>, *S.S. Papiha*<sup>3</sup>, *R. Chakraborty*<sup>2</sup>, *R. Deka*<sup>2</sup>. 1) Dept Human Sciences, Human Genetics Lab, Loughborough University, Loughborough, United Kingdom; 2) Department of Environmental Health, University of Cincinnati, Cincinnati, OH 45267. USA; 3) Institute of Human Genetics, University of Newcastle on Tyne. United Kingdom.

We have analysed genetic variation at 13 STR loci (CODIS core loci) in a sample of 18 ethnically and geographically diverse endogamous caste and tribal populations of the Indian subcontinent. A wide spectrum of allelic distribution at different loci was observed in different geographical and ethnic populations. Overall populations within geographical regions showed greater degree of similarity. Statistically significant differences were observed in a large number of inter-population comparisons. D18S51, FGA and D21S11 loci were the most polymorphic in all populations. FGA locus had the highest average heterozygosity (86%) and the lowest was observed for TPOX (69%). Average heterozygosity for all loci was 0.79. Coefficient of genetic diversity showed a narrow range for different loci (0.007 to 0.026) with an average of 1.4%, which indicates that these populations are at an early stage of micro-differentiation. Phylogenetic trees and principal component analysis computed from microsatellite allele frequencies provide support for socio-cultural and geographical assignment of these populations. Lowest match probability and highest exclusion probability was observed for the FGA locus in majority of the populations. Combined match probability was low (1 in  $1.55 \times 10^{-15}$  to 1 in  $7.47 \times 10^{-16}$ ), and combined exclusion probability was > 99.99%. There was no evidence of allelic association between loci studied, so these loci seem to comprise a suitable group of markers for population genetic purposes and for paternity and forensic testing. This research was supported by NIH grant GM45861, NIJ grant 98-LB-VX-002 and funds from Loughborough University.

**Principal Component Analysis for Selection of Optimal SNP-sets that Capture Intragenic Genetic Variation.** *B.D. Horne<sup>1,2</sup>, N.J. Camp<sup>1,2</sup>*. 1) Genetic Epidemiology, University of Utah School of Medicine, Salt Lake City, UT; 2) LDS Hospital, Intermountain Health Care, Salt Lake City, UT.

Association analysis results are often inconsistent across studies. A source of inconsistency can be the limited genetic data analyzed -usually one single nucleotide polymorphism (SNP) per gene. A solution would be to choose a set of SNPs which comprehensively covers the total genetic diversity of the gene. We introduce a novel method that defines sets of SNPs that comprise linkage disequilibrium (LD) groups and selects SNPs that encompass the intragenic genetic diversity. Principal component analysis (PCA) is performed on the SNP haplotypes in two stages. First, PCA is performed on all SNP haplotypes to determine the number of principal components (LD-groups) needed to describe the data. An LD-group is defined as a set of SNPs which all have substantial weighting in the same principal component. Second, PCA is performed on the SNP haplotypes for SNPs within each LD-group to establish an optimal set of genetic information content SNPs (gicSNPs) that sufficiently account for the diversity within each LD-group. The final set of gicSNPs will consist of at least one SNP from each LD-group, and should comprehensively cover the intra-genic diversity. This PCA method differs from other methods in that an LD-group need not consist of continuous DNA fragments or be mutually exclusive. As an example, we analyzed 19 SNPs in the ELAC2 gene spanning the gene and its promoter. Four LD-groups were identified in ELAC2 which accounted for 94% of the total variation. All except 2 very rare SNPs were represented in these LD-groups. Next, gicSNPs were chosen such that at least 90% of variation within each LD-group was maintained. The optimal gicSNP set included eight SNPs in total, 4 in the promoter region. Results of the PCA gicSNP method compared well with existing htSNP methods, but also provided advantages including better tagging of LD-groups, and quantification of the optimal number of SNPs to adequately describe a gene. This suggests that PCA may be useful in establishing optimal SNP sets which maximize the amount of genetic variation captured while using a minimal number of SNPs.

**Genetic variation at nine short tandem repeat loci in the Croatian populations.** *I. Martinovic Klaric<sup>1</sup>, M. Pericic<sup>1</sup>, L. Barac<sup>1</sup>, B. Janicijevic<sup>1</sup>, M. Kubat<sup>2</sup>, D. Pavicic<sup>1</sup>, R. Chakraborty<sup>3</sup>, L. Jin<sup>3</sup>, R. DeKa<sup>3</sup>, P. Rudan<sup>1</sup>.* 1) Institute for Anthropological Research, Zagreb, Croatia; 2) Department of Forensic Medicine and Criminology, University of Zagreb, School of Medicine, Zagreb, Croatia; 3) Division of Epidemiology and Biostatistics, Department of Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH, USA.

The aim of this study is to analyze genetic variation at nine autosomal short tandem repeat loci (D3S1358, vWA, FGA, THO1, TPOX, CSF1PO, D5S818, D13S317, D7S820) in six populations from Croatia, five of which are distributed in the islands of the eastern Adriatic coast and one is drawn from the mainland. The purpose is to investigate genetic structure of the Croatian populations and to evaluate the usefulness of the studied loci in regional level genetic differentiation. This investigation demonstrates a substantial genetic homogeneity of the Croatian gene pool as revealed by distribution of allelic frequencies, heterozygosity values, number of alleles and allele size variance, allele and genotype sharing data and the coefficient of gene differentiation. Even though a larger sample sizes for villages within islands could have provided a greater power of autosomal STRs in resolving patterns of microevolutionary structuring at regional level, the greatest discriminating power will certainly result when all classes of genetic markers, paternal, maternal and autosomal, will be jointly considered.

**The prevalence of methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms (C677T) and prothrombin 3-untranslated region variant (G20210A) in Mexican Cerebral Venous Thrombosis patients. A.**

*Jara, L. Martinez, H. Gonzalez, C. Cantu, A. Rasmussen, E. Alonso.* Genetics and Molecular Biology, Inst. Nal. Neurol. Neuroc., Mexico, City, Mexico, City, Mexico.

Cerebral venous thrombosis (CVT) is a serious disease, characterized by the severity of the clinical manifestations and the high mortality rate. It is a multifactorial disease caused by the interaction of acquired and genetic factors. CVT arises as a result of alterations in coagulation pathways. Recently, two genetic factors were described, the prothrombin gene variant and methylenetetrahydrofolate reductase polymorphism. A GA transition in position 20210 in the 3-UT region of the prothrombin gene is associated with elevated plasma prothrombin levels. The second factor described is a mutation (C677T) in the methylenetetrahydrofolate reductase (MTHFR) gene. MTHFR polymorphism is significantly associated with reduced enzyme activity and increased circulating levels of homocysteine. In this study, we examined the prevalence of prothrombin (G20210A) and MTHFR C677T gene variants in control subjects and in patients with CVT and determined the association between these variants and the presence of CVT. Fiftythree CVT patients and 456 healthy controls were recruited. Blood samples were collected for DNA analysis for the identification of the prothrombin and MTHFR gene (G20210A and C677T respectively) variants by PCR, followed by digestion with Taq I and Hinf I respectively. The resulting fragments were analyzed by electrophoresis and visualized with UV light. The frequency of the heterozygous variant G20210A was 1.5 % in the patients and 8.7 % in the controls, and the prevalence for the homozygous polymorphism MTHFR C677T was 16.4% and 12.7%, respectively. We also found an association between the prothrombin gene G20210A transition and the risk of CVT (OR=5.36, 95% CI=1.11-5.36). However, we did not find an association between MTHFR C677T mutation and the risk of CVT. These findings suggest that the G20210A variation could be a more important risk factor for CVT in Mexican population than the MTHFR mutation. However it would be important to increase the sample in order to support these findings.

**Beta globin haplotypes of three Mediterranean beta-thalassemia mutations observed in Mexican patients.** *B. Ibarra, M.T. Magaña, F.J. Perea.* Division de Genetica, Centro Invest Biomed Occte, Guadalajara, Jalisco, Mexico.

Beta thalassemia in Mexico has a wide spectrum of mutations, characteristic of the populations with low frequency of thalassemias, since to date 17 different mutations have been observed in 80 unrelated studied chromosomes. Mediterranean, Asian and rare alleles have been observed, the most common was GLN39TER (31.4%), followed by IVS1, G-A, +1 and IVS1, G-A, +110 (14.5% each). The beta globin haplotype (BH<sub>p</sub>) performed by RFLPs, was studied in 28 families with beta thalassemia, (13 with the mutation GLN39TER, 8 with IVS1, G-A, +1 and 5 with IVS1, G-A, +110), with the aim to search the genetic background of these alleles in Mexican population. All chromosomes with the IVS1, G-A, +1 mutation showed the same haplotype (1, + - - -), while for the 5 with the IVS1, G-A, +110 mutation, four had haplotype 1 and one haplotype 9 ( - - - - ), suggesting that its presence in Mexico has a single origin. On the other hand, the GLN39TER mutation showed a diversity of RFLP haplotypes since six were observed on haplotype 1, four with haplotype 2 ( - + + - +), two with haplotype 4 ( - - - - +) and one with haplotype 3 ( - + - + +). A great heterogeneity of BH<sub>p</sub>s with the GLN39TER mutation has also been observed in several Mediterranean countries like Algeria, which could be explained as a high mutation susceptibility at the second exon of the beta globin gene leading to a recurrent mutations on different chromosomal background.

**Correlation between apolipoprotein E and lipid levels in the three ethnic groups in Malaysia.** *W.T. Seet<sup>1</sup>, J.A. Tan<sup>2</sup>, S.Y. Tan<sup>1</sup>*. 1) Department of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia; 2) Department of Molecular Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

Apolipoprotein E, a polymorphic gene located on chromosome 19, has three common alleles: 2, 3 and 4. Studies have shown that individuals with at least one 2 allele tend to have lower cholesterol levels than those with homozygous 3 alleles and individuals with at least one 4 allele tend to have higher cholesterol levels than those with homozygous 3 allele. The objective of this project was to determine the correlation between apoE genotype and lipid levels (total cholesterol, triglyceride, high-density lipoprotein and low-density lipoprotein) in the Malay, Chinese and Indian populations in Malaysia. EDTA and plain blood were collected from 1127 blood donors. All of the subjects were screened for total cholesterol levels. Seventy nine subjects were screened for triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels. The ApoE gene sequence was amplified by the polymerase chain reaction (PCR) method and ApoE genotyping was performed by restriction enzyme digest with Hha I. One-way analysis of variance (ANOVA) was used to test the significance of total cholesterol, TG, HDL and LDL levels between the different apoE genotypes and gender. The results showed that there was a significant difference between the different apoE genotypes and total cholesterol levels in the Malay (n=345), Chinese (n=563) and Indian (n=219) populations with a p-value of 0.0001, 0.0001 and 0.009 respectively. Individuals with the E2/E2 and E2/E3 genotypes were found to have lower mean cholesterol levels than those with the E3/E4 and E4/E4 genotypes. No significance was found between gender and total cholesterol levels. Significant difference between the apoE genotypes and LDL levels in the 79 subjects (p=0.018) was also observed. However, no significance was found between genotypes and TG and HDL. Our study indicates that the apoE2 isoform is related to lower LDL and serum cholesterol levels whereas the apoE4 isoform tends to increase these values.

**Genetic structure of Sao Miguel's population (Azores) revealed by Y-Chromosome STR analysis.** *P.R. Pacheco, C.C. Branco, S. Costa, R. Cabral, T. Cymbron, B.R. Peixoto, L. Mota-Vieira.* Genetics & Molec Pathol Unit, Hosp Divino Espirito Santo, Azores Islands, Portugal.

The Azorean archipelago (Portugal) is located in the Atlantic Ocean, 1500 Km from the European mainland. The islands had no native population when Portuguese navigators first arrived from mainland in the XV century. Sao Miguel is the biggest and most populated (131,609 inhabitants) island of the Azores. In order to understand the genetic structure of Sao Miguel's population, we analysed seven Y-chromosome STR loci (DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392 and DYS393) in 100 unrelated individuals. Father's birthplace was used to select the samples: Nordeste (n=4), Povoacao (n=5), Vila Franca do Campo (n=8), Lagoa (n=11), Ribeira Grande (n=22) and Ponta Delgada (n=50), representing all the municipalities in Sao Miguel. The samples were typed by PCR with fluorescently labelled primers and products run in a CEQ8000 DNA Sequencer (Beckman Coulter). In a total of 100 complete seven-locus haplotypes, we observed 71 different haplotypes of which 54 appear only once. The mean heterozygosity per locus in the overall sample was 0.59, with the highest value in Nordeste (0.71) and the lowest in Ponta Delgada (0.56). On average the mean pairwise difference between two random haplotypes ranged from 4.49 in Ponta Delgada to 5.66 in Nordeste. The mean  $F_{st}$  value over all 7 loci was very low (-0.00758), denoting the absence of heterogeneity among the municipalities of the island. Pairwise genetic distances were computed for all six municipalities and no genetic heterogeneity was detected. AMOVA analysis shows that the percentage of variation attributable to differences within municipalities is extremely high compared to the differences between municipalities. These results provide evidence for reduced levels of Y-chromosome variability in the male lineages of Sao Miguel's population. We are currently analysing 4 SNPs (sY81, Tat, SRY1532 and SRY2627) and 1 indel polymorphism (YAP), in order to characterize the frequency and distribution of Y-chromosome haplogroups in the population of Sao Miguel. Funded by DRCT, Azores. (paulapacheco@hdes.pt).

**Genetic and environmental influences on racial/ethnic health disparities.** *N. Risch*<sup>1,2</sup>, *A. Karter*<sup>2</sup>. 1) Dept Genetics, Stanford Univ, Stanford, CA; 2) Division of Research, Kaiser Permanente, Oakland, CA.

The initiative Healthy People 2010 aims to reduce health disparities between racial/ethnic (R/E) groups. While Mendelian diseases typically show R/E variation, an unanswered question is the degree to which genetic versus non-genetic factors contribute to disparities in the prevalence of more common, complex diseases. To address this question, we considered a quantitative trait (QT) influenced by multiple additive loci and environmental factors. For discrete traits, we imposed a threshold model on the QT. Using published data on SNP frequencies and their differences in several R/E groups, we calculated how much difference could occur between R/E groups for average value of the QT and prevalence of the threshold trait. Model parameters included number of loci (N) and total heritability (H). We found that mean differences in QT between groups depends only on H and not N. The average difference in trait mean between R/E groups is about .6 standard deviations (sds) at high H (80%) and .3 sds at low H (20%). Similarly, prevalence differences were also a function of H but not N. For a 1% prevalence trait, the average prevalence ratio at high H was 2.3-3.0-fold, but only 1.2-1.5-fold at low H. These results suggest that genetic differentiation can play an important role in prevalence differences between R/E groups. Environmental factors, particularly R/E differences in access to preventive health care, are also clearly important. Here we compared R/E-specific disease prevalences for various diseases derived from the general California population to those from Kaiser Permanente (KP), a health plan offering uniform access to health care. We also examined R/E disparities in KP before and after adjustment for a wide range of environmental factors. For some disease outcomes, differences were attenuated with access to uniform care or after adjusting for environmental factors. In other cases R/E differentials persisted, suggesting residual factors, possibly genetic, are influential. Because no single piece of evidence is definitive, understanding the relative contribution of genes and environment to R/E health disparities requires a family of methodologic approaches.

**APOE distribution in World populations with new data from the Indian and the British populations.** *P. Singh<sup>1</sup>, M. Singh<sup>1</sup>, S.S. Mastana<sup>2</sup>*. 1) Dept Human Biololgy, Punjabi Univ, Patiala Punjab, India; 2) Human Sciences Department, Loughborough University, Loughborough, UK.

Human apolipoprotein E (APOE\*E) plays a major role in lipoprotein metabolism. Three common variants (\*E2, \*E3, & \*E4) of APOE gene show genetic variation. Epidemiological studies have found that the \*E4 allele is associated with longevity, increased cholesterol level, increased prevalence for cardiovascular and Alzheimer diseases. Apolipoprotein E genotypes (n =1065) were determined in four population groups (Punjabi Sikhs, Punjabi Hindus, Gonds and Koch) of India and three regionally sub-divided populations (Nottinghamshire, East Midlands and West Midlands) of UK. The extent and distribution of APOE allele frequencies were compared with 265 populations of the world. Three alleles APOE\*E2, APOE\*E3 and APOE\*E4 were observed with the contrasting variation. One interesting feature is low incidence or absence of \*E4 allele in many caste and tribal populations, even though cardiovascular diseases are relatively common. Higher values of \*E2 allele displayed similitude in Africa and Oceania (0.099 0.083 and 0.0990.057 respectively). Similarly highest value of \*E4 is sojourned in Oceania (0.2380.004) followed by Africa (0.1920.008). Intriguingly, coefficient of gene differentiation was found to be highest in South America (10.2%), though the highest gene diversity was observed in African populations (45.9%). APOE\*E2 revealed statistically significant decreasing cline towards North in Asia ( $r = -0.511$ ,  $y = -0.0013x + 0.1053$ ,  $df = 55$ ,  $p < .05$ ), which is not compatible with the coronary heart disease statistics in this continent. Correspondence analyses exhibited that Indians are in between European and Asian populations. Spatial autocorrelation analysis shows that the variation at this locus is influenced by isolation by distance with a strong positive correlation for lower distances upto 1313 kilometres. Large, intricate and unanticipated heterogeneity of this locus in its global perspective have insightful ramifications. The data presented also suggests that autochthon groups (like tribes) in India may throw better insight on the role of apolipoproteins in disease.

Program Nr: 1231 from 2003 ASHG Annual Meeting

**Recent natural selection on human populations detected by haplotype analysis of over 5,000 genes.** *G. Ruano, B.A. Salisbury, J.C. Stephens.* Genaissance Pharmaceuticals, New Haven, CT.

Intense interest exists in the detection of natural selection acting in the recent history of the human species. One reason for this interest is that polymorphisms currently or recently driven by natural selection may have important health consequences. Here we focus on a systematic, genome-wide approach to detecting gene variants that have been acted upon by geographically localized selection or by balancing selection. We introduce the use of gene-based haplotypes as a particularly sensitive resource for the detection of selection, and apply this analysis to haplotype data from over 5000 genes resequenced in 59 humans representing three continental populations (African, Asian, and European). A measure of genetic differentiation among populations,  $F_{ST}$ , is used to gauge which genes are most likely to have been under recent natural selection by their position within the empirical  $F_{ST}$  distribution. From this set of genes, we detail the findings for five whose haplotype  $F_{ST}$  values place them in the top 2.5% of the  $F_{ST}$  distribution, where one would expect to find haplotypes that had been favored by geographically-restricted natural selection. We also present a gene that may be a case of balancing selection, from the lower 2.5% of our empirical  $F_{ST}$  distribution. This empirical, gene-based haplotype approach is a promising strategy for identifying genes whose variation has been shaped in response to localized environmental challenges and genes whose variants are functionally distinct yet selectively co-maintained. Genes whose variation has been critical in the past are the lynchpins for a better understanding of human biology and our interactions with the world.

**Nucleotide diversity and linkage disequilibrium patterns in three regions marked by *Alu* insertion events. S.**

*Watkins*<sup>1</sup>, *J.A. Walker*<sup>2</sup>, *G.E. Kilroy*<sup>2</sup>, *M.A. Batzer*<sup>2</sup>, *L.B. Jorde*<sup>1</sup>. 1) Dept Human Genetics, Univ Utah, Salt Lake City, UT 84112; 2) Dept of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803.

To test hypotheses about the evolutionary dynamics of *Alu* insertions, we have characterized three unlinked genomic regions, each containing a polymorphic *Alu* insertion and nine to twelve closely-linked, common, single nucleotide polymorphisms (SNPs), in a panel of 152 Africans, 77 E. Asians, and 116 Europeans. As expected, chromosomes bearing the Ya5NBC148 (frequency [f] = 0.26) or Yb8NBC589 (f = 0.64) *Alu* insertions showed 45% and 26% reductions, respectively, in pairwise nucleotide diversity compare to non-*Alu* bearing chromosomes. In contrast, chromosomes containing *Alu* Yb8NBC120 (f = 0.52) showed a 41% increase in diversity. Age estimates for the *Alu* insertion event using closely linked microsatellite loci were 9 to 37 thousand and 88 to 394 thousand years ago for Ya5NBC148 and Yb8NBC589. In agreement with expectations, microsatellite variance was lower for haplotypes marked by the low frequency *Alu* insertion.

Haplotypes for a region of ~85 kb surrounding the Yb8NBC120 polymorphic *Alu* insertion were constructed using common SNPs that were 1) chosen randomly from the dbSNP database (8 loci) or 2) identified by resequencing of 21 ethnically diverse samples (9 loci). Haplotypes generated from dbSNP loci show higher diversity estimates for Europeans (2.51) than for Africans (2.20). In contrast, Africans show higher within-group diversity (2.81) than Europeans (2.07) when haplotypes were constructed using SNPs identified by resequencing. Elevated African diversity was also observed for the 2 other regions, where SNPs were also obtained by resequencing. These results suggest incomplete representation of human variation in the dbSNP database that may influence diversity estimates and haplotype construction for populations studies. For the 3 regions, *Alu*-bearing chromosomes did not show high levels linkage disequilibrium, possibly due to the age of the insertions or effects of the elements on recombination. Grant sponsorship: NIH GM59290.

**Genetic Structure of the population of Zulia State, Venezuela, based on Autosomal Hypervariable Loci.** *W. Zabala, L. Pineda, L. Borjas, E. Fernandez, C. Castillo, A. Socca.* Medical Genetic Unit, University of Zulia, Maracaibo, Zulia, Venezuela.

The genetic relationships between population groups can be studied through a comparison of their own genotypic and allele similarities. On this investigation, value is given to the utility of the microsatellite markers (STRs) for the study of the genetic structure of the population of Zulia. By Polymerase Chain Reaction under multiplex format, seven (7) autosomal systems were amplified into 688 different chromosomes of not-related individuals, from five populations of Zulia State: Maracaibo, Isla de Toas, San Jos de Heras, Bari y Yukpa. It was determined the allele and genotypic frequencies, Hardy-Weinberg equilibrium and other population estimators like the Wrights F-statistic, genetic distances and ethnic Admixture as well. It was proved that these markers were useful in the study of the genetic structure of the population in Zulia. It was shown that it does exist a clear genetic difference among these groups. The distribution of the genetic differences that were found, were in accordance with the history and behavior of each population. It was also proved the existence of a general tendency of not random crosses that causes a departure from panmictic in the studied population. The populations in Maracaibo and Isla de Toas showed a tri-racial origin, with a high European contribution, followed by the amerindian and with an African component in a lower value. The indigenous groups Bari and Yukpa showed exclusively an amerindian component and the San Jos de Heras an African one. The results of this investigation become a valuable tool for the molecular anthropology, the same, regional and world wide, providing important information about the origin and genetic comparison of the current population of Zulia.

**Genetic and Environmental Determination of Renal Function in Young Adult Twins.** *R. Vlietinck*<sup>1, 2</sup>, *M. Gielen*<sup>1</sup>, *R. Loos*<sup>2</sup>, *C. Derom*<sup>2</sup>, *S.J. Pinto-Sietsma*<sup>3</sup>, *M. Zeegers*<sup>1</sup>, *G. Beunen*<sup>4</sup>, *R. Fagard*<sup>5</sup>. 1) Population Genetics, Genomics & Bioinformatics, University of Maastricht, (Netherlands); 2) Human Genetics, University of Leuven, (Belgium); 3) Internal Medicine, University of Maastricht, (Netherlands); 4) Physical Education, University of Leuven, (Belgium); 5) Hypertension and Cardiovascular Revalidation, University of Leuven, (Belgium).

Genetic and environmental contribution of renal function was determined in 200 monozygous and 122 dizygous twinpairs, aged 18-34 years. Creatinin clearance (Ccr) was calculated from the creatinin in serum and 24-hour urine. It was also estimated with the Cockcroft-Gault formula. Genetic and environmental contribution was calculated using variance component analysis (VCA). Adjusted values were calculated from residuals by regressing on sex, age, lean bodyweight, smoking, glycemia and blood pressure.

The correlations of unadjusted calculated Ccr in MZ was higher ( $r_{MZ}=0.5$ ) than in DZ pairs ( $r_{DZ}=0.31$ ). These were significantly lower than of the estimated Ccr ( $r_{MZ}=0.77$ ,  $r_{DZ}=0.38$ ) and of the calculated Ccr after adjusting for the mentioned variables ( $r_{MZ}=0.89$ ,  $r_{DZ}=0.33$ ).

VCA showed that 20% of the unadjusted calculated Ccr was caused by genes, 26% by environment common to both twins and 54% by specific environment. For the estimated Ccr these were 80%, 0% and 20% respectively. After adjustment for the mentioned variables the genetic determination rose to 89%.

This study shows that the genetic determination of the estimated creatinin clearance is higher than that of the calculated clearance. After adjustment for the mentioned variables, the genetic contribution increased to 89% and the common environment disappeared. This shows that these variables account for most of the environmental determination of the Ccr. Accounting for them raises the heritability from 20% to 89%, which shows that this strategy may dramatically improve the chances of finding genes for renal function.

**Heritability of Prostate Related Phenotypes in Men of African Ancestry: The Tobago Family Prostate Study.**

*J.M. Zmuda<sup>1</sup>, C.M. Kammerer<sup>1</sup>, A.L. Patrick<sup>2</sup>, I. Miljkovic-Gacic<sup>1</sup>, D. Rodriguez<sup>1</sup>, C.H. Bunker<sup>1</sup>.* 1) Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 2) The Tobago Regional Health Authority, Scarborough, Tobago, Trinidad & Tobago.

Benign prostatic hyperplasia (BPH) is a prevalent condition with unknown etiology. Family history is an important risk factor for BPH suggesting a genetic component, but the genetics of BPH remains poorly defined, especially among men of African ancestry. In the present study, we determined the relative contribution of genetics and environmental factors to anatomical (prostate size), clinical (lower urinary tract symptoms) and biochemical (prostate specific antigen; PSA) surrogates of BPH in 287 Afro-Caribbean families consisting of 532 male siblings aged 38-89 years (meanSD; 5510 yrs). All men completed a digital rectal examination (DRE), serum PSA and were administered a questionnaire about the frequency of nocturia as part of a population-based prostate screening evaluation in over 3,000 Afro-Caribbean men. Men with a history of prostate cancer were excluded from the present analyses. Prostate volume was estimated from sagittal and transverse dimensions. Maximum likelihood methods were used to partition the variance of each prostate related phenotype into components attributable to measured covariates (age, height, weight, hypertension, diabetes, smoking) and additive genetic effects (heritability). MeanSD prostate volume and PSA were 2823 cubic centimeters and 1.61.4 ng/ml, respectively. Measured covariates accounted for 11% and 18% of the total variance in PSA and nocturia, but explained less than 1% of the variation in prostate size. PSA and nocturia demonstrated significant residual heritability ( $P < 0.01$ ). The heritability (meanSEM) of PSA and frequency of nocturia were 0.300.13 and 0.290.13. Our preliminary analyses suggest that there is a significant genetic component to clinical and biochemical measures of BPH among men of African descent. The high heritability of these prostate related phenotypes suggests that their inclusion in family-based studies might advance our understanding of the genetic mechanisms underlying BPH.

**Sequence Variation of Bradykinin Receptors B1 and B2 and association with hypertension.** *J. CUI<sup>1</sup>, E. Melista<sup>1,2</sup>, I. Chazaro<sup>1,4</sup>, Y. Zhang<sup>3</sup>, A.L. DeStefano<sup>3,4</sup>, A.J. Manolis<sup>5</sup>, C. Baldwin<sup>2</sup>, H. Gavras<sup>1</sup>.* 1) Hypertension Section, Boston University School of Medicine, Boston, MA; 2) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 3) Department of Neurology, Boston University School of Medicine, Boston, MA; 4) Department of Biostatistics, Boston University School of Public Health, Boston, MA; 5) Cardiology Division, Tzanio Hospital, Piraeus, Greece.

The two subtypes of Bradykinin Receptors B1 and B2 (BDKRB1, BDKRB2) mediate the function of Bradykinin, which is involved in the regulation of blood pressure and hence are attractive candidate genes for hypertension. To better understand the genetic variations in these genes, we sequenced a 64.4 kb genomic region containing the entire BDKRB1 and BDKRB2 in 16 hypertensive and 14 normotensive African American individuals. A total of 282 single-nucleotide polymorphisms (SNPs) were identified. We selected 18 SNPs for study in our complete cohorts, of African Americans, American Caucasians, and Greek Caucasians. Pairwise linkage disequilibrium (LD), measured by  $r^2$ , demonstrated a general pattern of decline with increasing distance. This pattern of decline did not vary among the three groups but we observed less LD in African Americans. The percent of  $r^2$  values larger than 0.1 in the three populations are 7.19%, 18.95% and 16.99% for African American, American Caucasian, and Greek Caucasian, respectively. We also evaluated 18 SNPs for differences in genotypic frequency between hypertensives and normotensives. One SNP in BDKRB1 and 3 SNPs in BDKRB2 were associated with hypertension status ( $p$ -values range between .026 and .0004) in American Caucasians, but not associated in African Americans or Greek Caucasians. Haplotypes were built using these 4 SNPs which were in high LD. There is a significant haplotype frequency difference between hypertensive and normotensives among our American Caucasian ( $p=0.035$ ) sample, but not in African Americans or Greek Caucasians. These results support the hypothesis that the African American population is an older population compared to the other samples and the two bradykinin receptors may play a role in blood pressure regulation.

**DNA typing from limited quantities of genomic DNA using whole genome amplification.** *G. Sun, R. Kaushal, M. Wolujewicz, D. Smelser, H. Cheng, M. Lu, L. Jin, R. Deka.* Dept Environmental Health, Univ Cincinnati, Cincinnati, OH.

Whole genome amplification (WGA) is a critical method for DNA analysis from limited quantities of genomic DNA. Several strategies for WGA have been developed during the past decade, each with variable fidelity, yield and coverage of the genome. In search for a reliable WGA method for typing VNTR, STR and SNP markers, we initially tested three popular methods, viz., degenerate oligonucleotide primed PCR (DOP), improved-primer extension preamplification (I-PEP) and multiple displacement amplification (MDA), typing the 13 CODIS STR loci. The results showed that both I-PEP and MDA have good coverage (13/13). However, DOP failed to provide satisfactory coverage with a dropout of 11 out of 13 loci. Since then, we have focused on I-PEP and MDA and typed an additional 33 dinucleotide repeat markers from the ABI linkage mapping (v2.5) panels 25, 26 and 27, the amelogenin locus, one VNTR and 19 SNP markers. The results show comparable coverage (67/67) using both methods, as well as fidelity, genotyping results completely matching with the results using native genomic DNA. Based on the amplification of the CODIS STR loci, we find that the yield of MDA is slightly higher than I-PEP (5 ng of input DNA). However, I-PEP products result in more uniform amplification. If the input DNA for WGA is too low or degraded, differential amplifications occur causing allelic imbalance. MDA products induce higher allelic imbalance. With an initial input amount of 5~50 ng of genomic DNA, both methods produce high fidelity DNA sufficient for 1000~1500 PCR amplifications. Genotype fidelity of 67 loci using both I-PEP and MDA products demonstrate that WGA methods can play significant role in DNA marker analysis from limited amount of native genomic DNA. Our analysis also demonstrates that blood spots on FTA cards are a good source of DNA suitable for both strategies.

**Bardet-Biedl Syndrome 1 and obesity in the Newfoundland population.** *W.S. Davidson<sup>1</sup>, Y. Fan<sup>1</sup>, P. Rahman<sup>2,3</sup>, L. Peddle<sup>2</sup>, D. Hefferton<sup>2</sup>, N. Gladney<sup>2</sup>, S.J. Moore<sup>3</sup>, J.S. Green<sup>3</sup>, P.S. Parfrey<sup>3</sup>.* 1) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada; 2) Newfound Genomics, 187 LeMarchant Road, St. John's, NF, Canada; 3) Faculty of Medicine, Health Sciences Centre, Memorial University, St. John's, NF, Canada.

Obesity is one of the primary clinical features of Bardet-Biedl Syndrome (BBS), a genetically heterogeneous disorder that is usually inherited as an autosomal recessive trait. It has been suggested that heterozygous carriers of BBS are predisposed to obesity. We set out to identify the common mutation in BBS1 families from southwest Newfoundland and to examine the relationship between this mutation and obesity in the general population. All affected members of the six Newfoundland BBS1 families were homozygous for the most common BBS1 mutation (M390R). BMIs were calculated for affected individuals, carriers, and non-carriers from these families. The affected individuals were more obese than the carriers ( $p < 0.05$ ) and the non-carriers ( $p < 0.01$ ) whereas the carriers and non-carriers are not statistically different ( $p > 0.25$ ). The contribution of the M390R BBS1 mutation to obesity in the general population was assessed by screening 200 obese individuals ( $BMI > 30 \text{ kg/m}^2$ ) and 200 ethnically matched, unrelated controls from the same region ( $BMI < 29 \text{ kg/m}^2$ ). The frequency of the M390R BBS1 mutation was identical in each group (0.0075). Our data do not support the hypothesis that BBS1 plays a significant role in the frequency of obesity in the general public in Newfoundland.

**Accuracy and Efficiency of Bayesian and EM Inference of Haplotypes in the Presence of Gene Conversion. R.**

*Adkins*<sup>1</sup>, *C. Campese*<sup>2</sup>, *R. Vaidya*<sup>2</sup>, *J. Krushkal*<sup>3</sup>, *T. Boyd*<sup>4</sup>. 1) Dept Pediatrics, Le Bonheur Hosp, Memphis, TN., USA; 2) Dept. Biology, Univ. Mass., USA; 3) Dept. Preventive Medicine, Univ. Tenn. Memphis, USA; 4) Dept. Pathology, Baystate Med. Center, USA.

Gene conversion creates a nonstandard pattern of correlation among nucleotide sites. Instead of patterns of linkage disequilibrium being simply the consequence of normal recombination, correlations in polymorphisms among sites are determined by the extent of gene conversion and the identity of the gene donating variants. Pituitary growth hormone (GH) is an excellent system within which to study the impact of gene conversion on computational inference of haplotypes. GH is one of 5 tightly-linked and highly-similar genes and is unusually polymorphic in that 27 SNPs occur in the span of 1,750 nucleotides. First we demonstrate that the high polymorphism and complex haplotype structure of GH is the consequence of recurrent gene conversion. Then, we compare the efficiency and accuracy of Bayesian and EM inferences of haplotypes. For validation of inferences we use an empirically determined set of haplotypes from a large independent study. Our results demonstrate that Bayesian inference of haplotypes as implemented by the program PHASE is superior to the most popular implementations of the EM algorithm when levels of polymorphism are high and gene conversion is present. Separately, we present evidence that polymorphism upstream of GH is associated with variation in term birth weight. This is a surprising finding given the accepted wisdom that pituitary GH is not involved in regulating fetal growth.

**Instability of Expanded CAG/CAA repeats in Spinocerebellar Ataxia Type 17.** *R. Gao<sup>1</sup>, M. Coolbaugh<sup>2</sup>, C. Zuhlke<sup>3</sup>, K. Nakamura<sup>4</sup>, A. Rasmussen<sup>5</sup>, T. Matsuura<sup>6</sup>, M. Siciliano<sup>2</sup>, T. Ashizawa<sup>1</sup>, X. Lin<sup>1</sup>.* 1) Neurology, University of Texas Medical Branch, Galveston, TX; 2) Department of Molecular Genetics, MD Anderson Cancer Center, Houston, Texas; 3) Institut für Humangenetik, Universität Lubeck, Lubeck, Germany; 4) Department of Neurology, The University of Tokyo School of Medicine, Tokyo, Japan; 5) Department of Neurogenetics and Molecular Biology, Instituto Nacional de Neurología y Neurocirugía, Mexico City, D.F. Mexico; 6) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas.

Expanding trinucleotide repeats are dynamic mutations causing many neurological conditions. Among the many factors that are implicated in repeat instability is the configuration of repeats: pure repeats are more prone to expand and/or contract than interrupted repeats. However, this hypothesis still lacks direct evidence. Here, we attempt to test the hypothesis in spinocerebellar ataxia type 17 (SCA17), which is caused by CAG repeat expansion coding for polyglutamine tract in TBP (TATA binding protein). SCA17 is unique among polyglutamine diseases in that the expanded CAG repeats in many patients are interrupted by CAA, and there are two types of repeat configurations: the simple configuration with long stretches of pure CAGs and the complex configuration containing CAA interruptions. By small pool PCR (SP-PCR) analysis of blood DNA from SCA17 patients of distinct racial backgrounds, we quantitatively assessed the instability of these two types of expanded alleles with similar length of repeat expansion. We observed that the mutation frequency in patients harboring pure CAGs is 2-3 folds of those having a complex repeat configuration. Interestingly, the less interrupted CAG repeats showed both expansion and deletion while more interrupted repeats exhibited mostly deletion. These data clearly demonstrated that repeat configuration is a critical determinant for instability in SCA17. Further studies of the underlying mechanisms will provide insights into understanding how the stability of repetitive sequences is maintained.

**Polymorphisms at VDR and DRD3 genes moderate the effect of lead exposure on postural balance in children.**

*B.M. Chakraborty, M. Wolujewicz, J. Mallik, K.N. Dietrich, A. Bhattacharya, R. Deka, R. Chakraborty.* Department of Environmental Health, University of Cincinnati, Cincinnati, OH.

Adverse effects of lead exposure, even at low levels, on neuromotor function have been observed in developing children. Also, some genes are known to moderate lead toxicokinetics. We investigated the possible involvement of several genes in inducing differential associations of lead exposure on neuromotor functions in developing children. In the Cincinnati Lead Study cohort, average blood lead level during the first 5 years of life (AvPb05) was taken as the measure of early exposure. Postural balance, an indicator of neuromotor function, was measured in four testing conditions, resulting in two variables, postural sway length, SL; and postural sway area, SA; in each testing condition. Height and age-adjusted log(SL) values were positively correlated with log AvPb05 ( $r = 0.249$  to  $0.260$ ;  $p < 0.001$ ) in all testing conditions, indicating a trend towards impaired postural balance with increasing blood lead levels in the 189 children examined. Neither blood lead level, nor any of the postural sway measures showed any significant difference by genotypes at the eight loci studied. However, the regression coefficients of log(AvPb05) on adjusted log(SL) showed significant differences ( $p < 0.01$ ) across genotypes of the DRD3 (Gly/Gly, Gly/Ser, and Ser/Ser) and VDR (TT, Tt, and tt) genes. For DRD3, the heterozygotes showed a significant positive regression effect, while for VDR, only the homozygous (TT, tt) children showed significant positive regression coefficients. This heterogeneity of regression coefficients represents a gene-environment interaction, and the pattern of the three-way relationship of genotype, blood lead, and postural sway measures suggests that the polymorphisms at DRD3 and VDR genes may moderate the effects of lead exposure on neuromotor functions in children. (Research supported by a pilot project of the NIEHS-funded program project P01-ES06096 awarded to the Center for Environmental Genetics at the University of Cincinnati).

**Pharmacogenetic association between 2AR haplotypes and lower bronchodilator responsiveness in Puerto Rican than in Mexican asthmatic subjects from the Genetics of Asthma in Latino Americans (GALA) Study.** *E.G.*

*Burchard<sup>1</sup>, S. Choudhry<sup>1</sup>, N. Ung<sup>1</sup>, K. Salari<sup>1</sup>, E. Ziv<sup>1</sup>, S. Nazario<sup>1</sup>, J. Casal<sup>2</sup>, J. Torres<sup>2</sup>, J. Rodriguez-Santana<sup>3</sup>, J. Senter Sylvia<sup>4</sup>, J. Salas<sup>5</sup>, M. Selman<sup>5</sup>, R. Chapela<sup>4</sup>, H.A. Boushey<sup>1</sup>, S.T. Weiss<sup>4</sup>, J. Ford<sup>6</sup>, D. Sheppard<sup>1</sup>, W. Rodriguez-Cintron<sup>2</sup>, J.M. Drazen<sup>4</sup>, E.K. Silverman<sup>1</sup>.* 1) University of California, San Francisco, San Francisco, CA; 2) University of Puerto Rico, San Juan, Puerto Rico; 3) Pediatric Pulmonary Program, Cardiovascular Center of Puerto Rico, San Juan; 4) Brigham and Womens Hospital, Boston, MA; 5) Instituto Nacional de Enfermedades Respiratorias, Mexico City, MX; 6) Harlem Lung Center, Harlem Hospital and Columbia University, NY.

Puerto Ricans (PR) and Mexicans (MX), represent the U.S. populations with the highest and lowest asthma prevalence, morbidity and mortality, respectively. We hypothesized that genetic variants in the beta 2 adrenergic receptor (2AR) gene contributed to differences in asthma and asthma severity. The Genetics of Asthma in Latino Americans (GALA) Study included 684 MX and PR asthmatic probands and their biologic parents (trios) recruited from Puerto Rico, Mexico and the U.S. We genotyped 430 trios and tested for association between 2AR haplotypes with asthma, asthma severity and bronchodilator responsiveness. Asthmatic Puerto Ricans had lower lung function, greater asthma morbidity, and longer asthma duration than asthmatic Mexicans. Puerto Ricans had on average 7.3% ( $p < 0.001$ ) lower bronchodilator reversibility in FEV1, higher risk of an Emergency Department visit in the previous year ( $OR = 2.63$ ,  $p < 0.001$ ), and of previous hospitalization for asthma ( $OR = 1.94$ ,  $p = 0.009$ ) than Mexicans. 2AR haplotypes were associated with asthma severity and bronchodilator responsiveness in Puerto Ricans ( $p = 0.01-0.001$ ) but not in Mexicans. Pharmacogenetic differences in bronchodilator responsiveness exist between PR and MX asthmatics. The 2AR locus may contribute to the observed differences in asthma prevalence, morbidity and mortality. These findings underscore the need for additional research on racial/ethnic differences in asthma morbidity and response to therapy.

**Human Pressor Responses: Heritability Estimations in White American Twins and Genotypic Associations at Adrenergic Loci.** *R. Chandran<sup>1</sup>, F. Rao<sup>1</sup>, D. King<sup>1</sup>, P. Cadman<sup>1</sup>, A. Chen<sup>1</sup>, K. Nguyen<sup>1</sup>, A. Bolotnikova<sup>1</sup>, G. Zhang<sup>2</sup>, B. Rana<sup>2</sup>, A. Joyner<sup>1</sup>, M. Robinson<sup>2</sup>, P. Insel<sup>1</sup>, N. Schork<sup>2</sup>, D. O'Connor<sup>1</sup>.* 1) Dept. of Medicine, UCSD and VASHDS, San Diego, CA; 2) Department of Psychiatry, UCSD, San Diego, CA.

It has been hypothesized that transient, repeated pressor responses may lead to long-term sustained hypertension in individuals at genetic risk. The Cold Pressor Test (CPT) consists of immersion of the non-dominant hand in ice water (0°C) for 1 minute, with continuous, non-invasive recording of BP and HR in the contralateral arm. Sensory afferent nerves relay the information to the hypothalamus. Vasoconstriction and an increase in HR & BP are mediated by adrenergic efferents. The Heart Rate (HR) response to cold stress is augmented in individuals with a genetic risk of hypertension, compared to family history negative normotensives. The study population was composed of 298 White Americans, 198 monozygotic (66%), and 100 dizygotic (34%) twin pairs. 48% of the participants had a positive family history of hypertension, while 52% did not. The average age of the population was 40 years, with a median of 45. Genotypic data for the ADRB1, ADRB2, and ADRB3 polymorphisms was available on a minimum of 189 individuals. The genetic analysis program SOLAR was used to compute heritability and the percentage of variability of the phenotype contributed by covariates. Age was the greatest contributing mechanism to variability in basal sbp, basal dbp, post cold pressor test sbp and dbp phenotypes in contrast to sex and family history of hypertension,  $H^2=37.81, 33.51, 31.11,$  and  $34.5$  respectively, with  $p=0.0000131, 0.0002779, 0.000293,$  and  $0.0000534$  respectively. Of the twelve ADRB polymorphisms used as covariates in this analysis, ADRB2 -654 and -47 made the greatest contribution to phenotypic variability. ADRB2 -654 contributed to 6.78%, 5.01%, and 5.65% to the variability of basal dbp, post test hr, and change in sbp. ADRB2 -47 contributed 5.53% to the change in sbp. Human twin heritability computations suggest that cardiovascular responses (blood pressure and heart rate) to an environmental stressor (cold stress test) are heritable.

**Variation in the Resistin Gene is Associated with Diabetes-Related Phenotypes in Finns.** *K.N. Conneely<sup>1</sup>, K. Silander<sup>2</sup>, K.N. Lazaridis<sup>3</sup>, K.L. Mohlke<sup>3</sup>, L.J. Scott<sup>1</sup>, T.T. Valle<sup>2</sup>, R.M. Watanabe<sup>4</sup>, T.A. Buchanan<sup>4</sup>, R.N. Bergman<sup>4</sup>, J. Tuomilehto<sup>2</sup>, F.S. Collins<sup>3</sup>, M. Boehnke<sup>1</sup>.* 1) U. Michigan, Ann Arbor, MI; 2) Nat'l Public Health Inst., Helsinki, Finland; 3) NHGRI, Bethesda, MD; 4) U. Southern California, Los Angeles, CA.

Resistin is a peptide hormone produced by adipocytes that is present at higher levels in obese mice and may be involved in glucose homeostasis, possibly through regulation of insulin sensitivity. Human studies have found associations between polymorphisms of the resistin gene and BMI, insulin-related variables, and blood pressure. Associations between resistin and type 2 diabetes (T2D) have been reported, but not in all studies. The aim of the Finland-U.S. Investigation of NIDDM Genetics (FUSION) Study is to identify genetic variants that contribute to the development of T2D. We analyzed 781 T2D cases and 409 controls for association between resistin and T2D and related traits. Three previously identified common, non-coding SNPs were typed: one from the promoter region (-420CG) and two from intron 2 (+156CT, +298GA). The three SNPs were in strong linkage disequilibrium and formed two main haplotypes: CCG (76.9%) and GTA (21.6%). We found no significant association between the SNPs and T2D ( $p = .55$ ), but did find associations with T2D-related phenotypes. For cases, the number of copies of the CCG haplotype was positively associated with weight-related traits. Mean BMI was 29.0, 29.6, and 30.4 kg/m<sup>2</sup> for cases with 0, 1, or 2 copies of CCG ( $p = .025$ , all tests based on age-sex adjusted additive effects of haplotypes on transformed variables.) Mean waist circumference was 99.5, 101.0, and 103.9 cm for cases with 0, 1, or 2 copies of CCG ( $p = .001$ ). Positive associations were also found for weight ( $p = .006$ ), weight change since age 20 ( $p = .014$ ), and waist-hip ratio ( $p = .022$ ). We saw similar results for individual SNPs. For controls, the C allele of SNP -420CG was associated with lower insulin sensitivity ( $p = .008$ ) and higher acute insulin response ( $p = .017$ ) assessed by intravenous glucose tolerance test. Our results provide evidence that resistin is associated with variation in weight, fat distribution, and insulin resistance.

**Association Between Frizzled Related Protein Polymorphisms and Osteoporosis Phenotypes in Older Women.**

*W.-C. Hsueh<sup>1</sup>, J.M. Zmuda<sup>2</sup>, L. Lui<sup>1</sup>, K.L. Stone<sup>1</sup>, J.A. Cauley<sup>2</sup>, T.A. Hillier<sup>3</sup>, J. Li<sup>4</sup>, R. Higuchi<sup>4</sup>, D.R. Greene<sup>4</sup>, S. Germer<sup>4</sup>, B. Fijal<sup>4</sup>, S. Kwon-Ro<sup>4</sup>, J. Mangacat<sup>4</sup>, N. Umblas<sup>4</sup>, J.D. Allard<sup>5</sup>, T. Nikolcheva<sup>5</sup>, G. Peltz<sup>5</sup>, S.R. Cummings<sup>1</sup>.*  
1) UCSF, San Francisco, CA; 2) University of Pittsburgh, Pittsburgh, PA; 3) Kaiser Permanente Center for Health Research Northwest/Hawaii, Portland, OR; 4) Roche Molecular Systems, Alameda, CA; 5) Roche Palo Alto, Palo Alto, CA.

Frizzled related protein (FRZB) acts as a soluble Wnt-binding protein that antagonizes Wnt signaling, a secreted growth factor implicated in skeletal morphogenesis. Our recent studies showed that FRZB is involved in the determination of bone mineral density (BMD) in mice and a single nucleotide polymorphism (SNP) (Arg324Gly) is associated with vertebral fracture among Caucasian women aged 65 years or older in the Study of Osteoporotic Fractures. Here we report results from studies investigating association between multiple SNPs in this gene and osteoporosis phenotypes using a case-control study design. A total of 9 SNPs (denoted as SNP1-9, the Arg324Gly SNP is SNP8) were typed in 51 cases with hip fracture and lowest 5% BMD, 262 cases with incident vertebral fracture, and 276 cases with low hip BMD (T score < -2.5). These case groups were compared to 278 fracture-free controls with normal BMD. All but 1 SNP are in untranslated regions. In <sup>2</sup> tests using single SNPs, there was a weak association between SNP1 (near exon 3) and fractures (hip and vertebral) (p=0.04-0.09). SNP9, which is in tight linkage disequilibrium with SNP8, also showed an association with vertebral fracture (p=0.01). There was no association between any other SNP and fractures, nor was there association between any SNP and low hip BMD. Haplotype analyses using various SNP combinations also supported findings using single SNPs. Only haplotypes containing SNP1 were associated with hip fracture (p=0.02-0.06), but the same haplotypes were not associated with vertebral fracture. We conclude that FRZB variants may play a role in osteoporosis, and that different allelic variants may influence clinically different manifestations of osteoporosis. Confirmation studies using a larger sample are underway.

**Social functioning in male premutation carriers of Fragile X (FRAX) syndrome.** *A.S. Rigby<sup>1</sup>, J. Turk<sup>2</sup>, A.C. Mills<sup>2</sup>, N. James<sup>3</sup>, C. Hollis<sup>3</sup>, K. Cornish<sup>4</sup>, A. Dalton<sup>5</sup>, T. Manly<sup>6</sup>.* 1) Children's Hospital, University of Sheffield, Sheffield, S Yorkshire, UK; 2) Department of Clinical Development Sciences, St Georges Hospital Medical School, London, UK; 3) Division of Psychiatry, University of Nottingham, Nottingham, UK; 4) Department of Education and Counselling Psychology, McGill University, Montreal, Canada; 5) Department of Molecular Genetics, North Trent Molecular Genetics Service, Sheffield UK; 6) MRC Cognition and Brain Sciences Unit, Addenbrookes Hospital, University of Cambridge, UK.

Previous small studies suggest males with FRAX premutation may show a phenotype similar to full mutation males. We assess if such males show phenotypic features similar to high functioning autism/Asperger syndrome. We report a large national UK study of adult premutation carriers recruited through regional genetics centres and the British Fragile X Society. Each case was IQ and age matched to 1 familial and 1 non-familial control. To date we have recruited 42 premutation males, 14 familial and 24 non-familial controls. All completed a new self-assessment screening test, the Autism-Spectrum Quotient (ASQ) consisting of 5 subscales; social skills, attention to detail, communication, attention switching and imagination. High scores imply poor performance. Premutation males scored highest on the ASQ (mean 20.2). Non-family controls scored the lowest (15.3). Family controls were intermediate (19.3). Subscale analysis showed no significant differences between premutation males and family controls. However, both groups had significantly higher scores than non-family controls on the attention switching and communication subscales. Premutation males also had significantly higher scores than non-family controls on the imagination subscale. These findings indicate that premutation males are more impaired than the general population in terms of the total ASQ score and in particular with attention switching. However, a familial tendency cannot be excluded given the intermediate performance of the family controls. The findings are consistent with premutation FRAX males having specific developmental difficulties with social and communication skills as well as attention skills.

**Study of apolipoprotein E genotypes in end-stage renal failure patients using a rapid molecular screening technique.** *S.Y. Tan<sup>1</sup>, W.T. Seet<sup>1</sup>, J.A. Tan<sup>2</sup>*. 1) Department of Medicine, University of Malaya, Kuala Lumpur, Malaysia; 2) Department of Molecular Medicine, University of Malaya, Kuala Lumpur, Malaysia.

In end-stage renal failure (ESRF), lipid abnormalities are frequently observed. Abnormal lipid metabolism may play an important role in the progression of renal disease. Apolipoprotein E2 (ApoE2), a variant of the common apoE3 caused by a change of residue 158 from Arg to Cys, has a lower affinity for the LDL receptors (less than 5% of apoE3) and the apoE receptors. Therefore they are cleared at a slower rate compared to apoE3 and thus contribute to the accumulation of remnant particles in the plasma, which is derived from partial catabolism of triglyceride-rich lipoproteins. In this study, the association of apolipoprotein E polymorphism with renal failure, especially the apoE2 genotype, was investigated in 123 end-stage renal failure patients. EDTA blood was collected from ESRF patients at the Renal Unit of University of Malaya Medical Centre. Characterization of the apoE variants was carried out by a rapid molecular screening method consisting of DNA amplification followed by restriction enzyme digestion using *Hha* I. Digested products were electrophoresed on 3% Molecular Screening Agarose to separate the DNA fragments. The restriction enzyme digested fragments were observed as distinct and well separated bands on MS Agarose and thus proved the horizontal gel system to be an efficient electrophoresis medium for the separation and detection of the six apoE genotypes. The frequencies of the three apoE alleles (2, 3 and 4) from our diseased population were found to be 0.037, 0.865 and 0.098 respectively. The allele frequency for the control population (n=123) was found to be 0.077 (p=0.080), 0.825 (p=0.262) and 0.098 (p=0.879) respectively. The results indicated that the frequencies of the three apoE alleles from control individuals were almost identical to the ESRF patients with exception of the 2 allele which was slightly lower in the ESRF population. Our study found no correlation between apoE alleles and ESRF. The slight decrease in the 2 allele found in the ESRF population in this study is not significant enough (p=0.080) to be conclusive.

**APOLIPROTEIN E GENOTYPING IN PATIENTS WITH ALZHEIMERS DISEASE IN RISARALDA, COLOMBIA, SA.** *L. Rengifo, M.Y. Sánchez, P.A. Osorio, R.P. Alarcón, L.H. García, J. Rodríguez, M. Castañeda, H. Serrano, A.H. Alegría, Centro de Biología Molecular y Biotecnología-CENBIOTEP. Basic Sci, Fac Med, Favi II, Univ Technologica de Pereira, Pereira, Risaralda, Colombia, SA.*

Apolipoprotein E isoforms have been associated with different risks for late onset Alzheimers disease (AD). Individuals with the 4 allele have a higher probability of acquiring the disease and the age for the appearance of the first symptoms is an earlier one. The 2 allele seems to have a protective effect. In Colombia, SA, no studies have been carried out to determine if these associations are still valid. A study was performed on a group of patients with clinically diagnosed Alzheimers disease and a comparison made with the results obtained for a normal population in the Department of Risaralda, Colombia SA. A fragment of the ApoE gene corresponding to 244 bp was amplified by PCR. The fragment was later digested with HhaI for distinguishing the 3 alleles. 54 patients (22 men and 32 women, 40.7% and 59.3% respectively) with ages between 49 and 92 years were genotyped. A group of 654 normal people was used as a control. In the AD group the 4 allele had a relative frequency of 18.5% while the control group had 8.93%, a result statically significant ( $P=0.022$ ). The 4 allele was more frequent in males than in females (10.0% and 8.0%). The 4/3 genotype was present in the AD group with 22% and 14.07% in the control group. The 4/4 genotype was 5.5% in the AD patients and 1.19% in the control group. The most frequent allele was 3 with 79.63% in the AD group and 83.78% in the control. The 3/3 genotype was present in 68.5% and 72.5% respectively. No allele correlation was detected with age or the initiation of symptoms.

**Association of the AGT 174 and 235 Polymorphisms with Blood Pressure in Ghana.** *M. Robinson*<sup>1</sup>, *S. Williams*<sup>2</sup>. 1) Univ California, San Diego, La Jolla, CA; 2) Vanderbilt Univ, Nashville, TN.

Genetic analysis of hypertension has revealed complex and inconsistent results, making it difficult to draw clear conclusions regarding the impact of specific genes on blood pressure regulation in diverse human populations. Using a different approach, we examined the contributions of variations in two Angiotensinogen (AGT) polymorphisms, in a monogenic and polygenic analysis, in conjunction with the age of the subjects, to assess their association with blood pressures. A total of 177 subjects (51 normotensives and 126 hypertensives) were recruited in Accra, Ghana. Blood pressures were determined using an automatic blood pressure reader on the right arm in a seated position. Blood samples were collected, the DNA was isolated and genotyped for each of the AGT T174M and M235T polymorphisms using standard PCR/RFLP methods for these polymorphisms. In our analysis, the AGT polymorphisms were the independent variables, blood pressure measurements were the dependent variables, and age was assigned as the covariate. We observed a significant association of the AGT T174M polymorphism with average systolic and diastolic blood pressures ( $p=0.000$ , and  $p=0.010$ , respectively). Also, we observed a significant association of the AGT M235T polymorphism with systolic and diastolic blood pressures ( $p=0.000$  and  $p=0.014$ , respectively). Finally, we observed a simultaneous significant association of the AGT T174M and M235T polymorphisms with systolic and diastolic blood pressure ( $p=0.000$ , and  $p=0.026$ , respectively). Age was used as a covariate in all models. Our results indicate that combinations of alleles at these two AGT sites, in conjunction with age, may be significantly associated with elevated systolic and diastolic blood pressures. Also, our data demonstrate the utility of the monogenic and polygenic analysis as a method of analyzing blood pressure variation. We suggest that inconsistent results from previous association studies on the Angiotensinogen polymorphism with blood pressure regulation in African derived populations may have resulted from an incomplete model in the different study populations.

**HEREDITARY HEMOCHROMATOSIS: GENOTYPE/PHENOTYPE CORRELATIONS FOR THE MAIN HFE1 MUTATIONS.** *V. Scotet<sup>1</sup>, MC. Merour<sup>2</sup>, AY. Mercier<sup>2</sup>, B. Chanu<sup>2</sup>, T. Le Faou<sup>3</sup>, O. Raguene<sup>1</sup>, G. Le Gac<sup>1</sup>, C. Mura<sup>1</sup>, JB. Nousbaum<sup>4</sup>, C. Ferec<sup>1,2</sup>.* 1) Dept de Genetique Moleculaire, INSERM EMI 01-15, Brest, France; 2) EFS-Bretagne, Brest, France; 3) EFS-Bretagne, Quimper, France; 4) Dept d'Hepato-Gastroenterologie, Brest, France.

Characterized by iron overload, hereditary hemochromatosis (HH) is the most common autosomal recessive disorder in Caucasian populations. A candidate gene for HH was cloned in 1996 (HFE1). It includes a main mutation (C282Y), two susceptibility factors (H63D, S65C) and a tenth of private mutations. HH presents a large genetic and allelic heterogeneity, and the aim of this study was to describe the phenotypic expression of the main HFE1 genotypes. We retrospectively analyzed a cohort of HH patients from western Brittany, France. A clinical questionnaire, completed at the time of onset, provides data on biochemical and clinical signs, genotype and treatment. This study included 585 patients: 433 C282Y/C282Y, 114 C282Y/H63D and 38 H63D/H63D. The age at onset was significantly lower in the two last groups (50.7 y. and 52.4 y. vs. 47.9 y. -  $p=0.02$ ). Serum iron, ferritin and transferrin saturation levels were also significantly lower in those two groups ( $p<0.001$  for each parameter). The C282Y/H63D and H63D/H63D genotypes were associated with lower frequency of arthritis (26.7% and 25.7% vs. 44.0% -  $p=0.0012$ ) and of skin pigmentation (3.7% and 13.9% vs. 24.0% -  $p<0.0001$ ) in comparison with the C282Y/C282Y genotype. Moreover, higher proportions of metabolic disorders (41.2% and 56.7% vs. 26.5% -  $p<0.0001$ ) and of high blood pressure (29.2% and 34.4% vs. 19.4% -  $p=0.02$ ) were observed in the patients carrying those genotypes. The comparison of the C282Y/H63D and H63D/H63D genotypes showed no significant difference, excepted a significantly higher proportion of skin pigmentation among the H63D-homozygous patients (13.9% vs. 3.7% -  $p=0.028$ ). This study highlights the correlation between HFE1 genotypes and phenotype in HH. The results confirm that the two less common genotypes are associated with a milder form of HH, and show that the patients carrying those genotypes present more frequently metabolic disorders.

**Association of a regulatory intronic polymorphism in the *PDCDI* gene with the risk of sporadic systemic lupus erythematosus and the occurrence of antiphospholipid antibodies.** *D.K. Sanghera*<sup>1</sup>, *S. Manzi*<sup>2</sup>, *F. Bontempo*<sup>2</sup>, *C. Nestlerode*<sup>1</sup>, *M.I. Kamboh*<sup>1</sup>. 1) Department of Human Genetics, GSPH, University of Pittsburgh, Pittsburgh, PA; 2) Department of Medicine, University of Pittsburgh, Pittsburgh, PA.

Systemic lupus erythematosus (SLE) is a chronic multifactorial autoimmune disease associated with diverse clinical manifestations. Although a significant amount of genetic component is involved in the etiology of SLE, the precise role of specific genes is yet to be identified. A regulatory polymorphism in intron 4 (G/A) of the programmed cell death 1 (*PDCDI*) gene has recently been shown to be associated with SLE risk in familial and sporadic cases from European, European American and Mexican origin. In this investigation we examined the role of this polymorphism in a biracial case-control cohort from Pittsburgh comprising 276 US White and 35 African American SLE patients and age matched healthy 359 US White and 31 African American controls. The frequency of the A allele was significantly higher in US White controls than African American controls (0.107 vs. 0.048;  $p=0.046$ ). There was no significant difference in the frequency of the A allele between SLE cases and controls in the white (0.107 vs. 0.129;  $p=0.84$ ) and black (0.048 vs. 0.100;  $p=0.25$ ) cohorts. However, a regression model including antiphospholipid antibodies (APA) as a covariate revealed that both APA ( $p=0.00001$ ) and *PDCDI* genotype ( $p=0.0385$ ) were independent risk factors for SLE. APA-adjusted odds ratio (OR) between A allele carriers (AA + AG genotypes) versus the GG genotype showed modest association in whites (OR = 1.52, 95% CI: 1.12 1.92;  $p=0.039$ ) and African Americans (OR = 2.91, 95% CI: 1.52 4.31;  $p=0.139$ ). Furthermore, we observed that the A allele carriers were associated with protection against the occurrence of APA in both controls (OR = 0.339, 95% CI: 0.19 0.82;  $p=0.0098$ ) and SLE cases (OR = 0.57, 95% CI: 0.32 1.01;  $p=0.054$ ). In conclusion, our data indicate that the *PDCDI* intron 4 polymorphism affects the occurrence of antiphospholipid antibodies and that it may slightly modify the risk of sporadic SLE.

**Increased risk of idiopathic chronic pancreatitis in cystic fibrosis carriers.** *J. Yan*<sup>1</sup>, *J. Cohn*<sup>2,3</sup>, *J. Neoptolemos*<sup>4</sup>, *J. Feng*<sup>1</sup>, *Z. Jiang*<sup>1</sup>, *W. Greenhalf*<sup>4</sup>, *I. Ellis*<sup>5</sup>, *S. Sommer*<sup>1</sup>. 1) Dept Molecular Genetics, City of Hope, Duarte, CA; 2) Duke Univ., Dept. of Medicine, Durham, NC; 3) V.A. Med. Ctr., Durham, NC; 4) Univ. of Liverpool, Dept. of Surgery, Liverpool, UK; 5) Alder Hey Children's Hosp., Liverpool, UK.

Idiopathic chronic pancreatitis (ICP) is associated with having mutations in both copies of CFTR, the CF gene. In this study, 52 ICP patients were comprehensively tested for rare CFTR mutations. This analysis detected common CF-causing mutations in 8 patients. Seven patients were CF carriers who had a normal second CFTR allele (4.8 times expected frequency,  $p < 0.001$ ). Thus, ICP differs from other CFTR-related conditions in that it is associated with CF carriers who have one documented normal CFTR allele. CF carriers are frequent. For ICP, the data suggest that carriers of one CF mutation have a higher attributable risk, while compound heterozygotes for a CF-causing mutation and another milder mutation have a higher relative risk.

**The Roles of Selection and Demography in Shaping Patterns of Variation in Vitamin D-Binding Protein.** *M. Pilkington, M.F. Hammer.* The Genomic Analysis and Technology Core, The University of Arizona, Tucson, AZ.

Relatively few genes have been identified in humans to be the targets of natural selection in response to environmental challenges. This work focuses on examining patterns of genetic variation at vitamin D-binding protein (DBP). DBP is a critical component of the vitamin D synthesis, metabolic, and activation pathway. The three major DBP alleles, termed Gc\*1F, Gc\*1S and Gc\*2, are distributed clinally across all continents, and their frequencies appear tightly correlated to skin pigmentation and latitude. The vitamin D hypothesis posits that humans have developed dark skin near the equator to protect from the over-absorption of vitamin D, and lighter skin further from the equator to protect from the under-absorption of vitamin D. Therefore, it is hypothesized that the vitamin D pathway is tightly regulated to maintain calcium homeostasis in humans. The goal of this study is to further characterize geographic patterns of DBP polymorphism to test hypotheses about the relative roles of demography and natural selection in shaping patterns of variation at this locus. Partial correlations between skin pigmentation, Gc allele (1F,1S and 2), and latitude were generated and Pearson's r statistic calculated for approximately 30 globally distributed populations (N=6983). Partial correlations between skin pigmentation and DBP allele frequency, holding latitude constant, indicated marginal statistical significance for the 1F and 1S alleles ( $p=0.072$  and  $0.071$ , respectively), but not for the 2 allele ( $p = 0.375$ ). In contrast, partial correlations between latitude and allele frequency, holding skin pigmentation constant, were not statistically significant; while correlations between latitude and skin pigmentation, holding allele frequency constant, were highly statistically significant (i.e.,  $p < 0.001$ ). Additionally, the residuals from plots of allele frequency against latitude, and skin pigmentation against latitude (which are not expected be correlated if allele frequencies are distributed randomly) result in highly significant correlations. These analyses indicate complex interactions between various components of the vitamin D synthesis and activation pathway.

**MC1R gene, nevus phenotypes and sun-related covariates modify CDKN2A penetrance in french melanoma-prone families.** *V. Chaudru*<sup>1</sup>, *A. Chompret*<sup>2</sup>, *A. Minire*<sup>2</sup>, *K. Laud*<sup>2</sup>, *M.F. Avril*<sup>2</sup>, *B. Bressac-de Paillerets*<sup>2</sup>, *F. Demenais*<sup>1</sup>. 1) INSERM EMI 00-06, Evry, France; 2) Institut Gustave Roussy, Villejuif, France.

Germline mutations in the cyclin-dependent kinase inhibitor gene, CDKN2A, are melanoma-predisposing alleles that confer high but incomplete penetrance, suggesting risk modification by other genetic and/or environmental factors. Variants of melanocortin-1 receptor gene, MC1R, have been shown to be associated with red hair, fair skin as well as melanoma independently of skin type. Other factors including nevus phenotypes (total number, dysplastic nevi), pigmentary traits (skin, hair and eye color), skin reactions to sunlight and degree of sun exposure, also influence melanoma risk. Our goal was to examine the joint effects of MC1R variants (R151C, R160W, D294H), nevus phenotypes, pigmentary traits and sun-related covariates on CDKN2A penetrance. Clinical, genetic, and covariate data were recorded in 53 French melanoma-prone families of which 20 had co-segregating CDKN2A mutations. Analysis of the co-transmission of CMM and CDKN2A alleles was conducted by likelihood based-methods using the regressive logistic models which can account for a variation of disease risk with age and can include the aforementioned risk factors as covariates. Tests for significant risk factors were conducted using a stepwise procedure. The factors that influence significantly CMM risk in carriers and non-carriers of CDKN2A mutations, in the whole family sample, include sunburns (OR=4.7), MC1R variants (OR=2.3), number of nevi (OR=2.1), dysplastic nevi (OR=1.8), sun exposure (OR=1.7) and fair skin (OR=0.8). In the subset of 20 families with CDKN2A mutations, the three significant factors modifying CDKN2A penetrance are: sunburns (OR=3.97), dysplastic nevi (OR=3.49) and MC1R variants (OR=2.64). In CDKN2A mutation carriers, the cumulative risk of melanoma is 0.58 by age 80 years and reaches 0.97 by adding presence of MC1R variants and 1.0 with dysplastic nevi or history of sunburns. This study shows that several pathways (cell-cycle pathway, nevus pathway, UV-sensitivity pathway, and cell-signaling pathway) are likely involved in the development of malignant melanoma.

**The influence of HLA on acute lymphoblastic leukemia of childhood.** *W. Klitz<sup>1</sup>, E. Trachtenberg<sup>1</sup>, M. Maiers<sup>2</sup>.* 1) Children's Hospital Oakland Research Institute, Oakland, CA; 2) National Marrow Donor Program, Minneapolis, MN.

Acute lymphoblastic leukemia of childhood (cALL) is the most common childhood cancer and the most common single disease for which bone marrow transplantation is employed, yet few clearly established etiological factors are known. Genetic predisposition according to HLA type might suggest an underlying infectious etiology. With this context in mind, we examined a large series of cALL patients of European American background drawn from the National Marrow Donor Program (NMDP) database. Two controls for each of 1,904 cALL patients, drawn from the NMDP donor registry, were matched for ethnicity and year of HLA typing from 1997 to 2002. Despite the limitations of low resolution DNA typing performed on the HLA A, B and DRB1 loci, and serological typing on a portion of the HLA class I typings, strong protective and predispositional effects were evident at both the HLA class I and class II regions. Among the 15 HLA A alleles recorded in the sample, the alleles A\*11, A\*24 and A\*30 were each predisposing, while A\*01 was associated with disease protection. Of 28 HLA B locus alleles only protective effects could be demonstrated, including the alleles B\*07, B\*08, B\*44, B\*62, B\*14, B\*60 and B\*57. At the 12 low resolution alleles examined at the DRB1 locus, DRB1\*03 was protective and DRB1\*11 was predisposing. The common extended HLA haplotype A\*01-B\*08-DRB1\*03 is the single best explanation for the protective effects of each of the constituent alleles. Further analysis revealed the allele B\*08 is most closely identified with the protective effect of the haplotype. It was possible to identify several additional genetic effects operating at the genotypic level. We conclude that the substantial impact of HLA on cALL predisposition deserves further examination in order to determine its implications for a role of pathogens or immune surveillance in the disease.

**Genetic linkage and association of single nucleotide polymorphisms in the IL10 region and HCV clearance.** T.K. Oleksyk<sup>1</sup>, C.L. Thio<sup>2</sup>, A.L. Truelove<sup>1,3</sup>, J.J. Goedert<sup>4</sup>, S. Donfield<sup>5</sup>, D.L. Thomas<sup>2,6</sup>, S.J. O'Brien<sup>1</sup>, M.W. Smith<sup>1,3</sup>. 1) Laboratory of Genomic Diversity, NCI, Frederick, MD; 2) Department of Medicine, Johns Hopkins University, Baltimore, MD; 3) Basic Research Program, SAIC, NCI, Frederick, MD; 4) Viral Epidemiology Branch, NCI, Bethesda, MD; 5) Rho, Inc., Chapel Hill, NC; 6) Department of Epidemiology, Johns Hopkins University, Baltimore, MD.

Hepatitis C virus (HCV) is an infectious blood-borne pathogen that is infrequently cleared from the host (approximately 15% of the time) while usually persisting as a chronic infection. Host differences are likely critical in determining HCV clearance, and showing their involvement will give insights into HCV pathogenesis. The inflammatory response is crucial in HCV infection and previous reports have implicated interleukin 10 (*IL10*) polymorphisms with successful anti-HCV therapy; however they have not been studied in natural viral clearance. *IL10* is a cytokine synthesis inhibitory factor that balances TH1 and TH2 immune response, and is involved in many aspects of human disease. We tested 67 SNPs in the *IL10* region, which included the *SRGAP2*, *IKKE*, *NORE1*, *MAPKAPK2*, and *PIGR* genes along with the *IL10* paralogs *IL19*, *IL20* and *IL24*, for association with HCV clearance versus persistence during infection. 32 SNPs were sampled within *IL10*, and 35 more from the surrounding 300kb region. Of those, 38 had minor allele frequencies 3%. A total of 274 African Americans (91 clearance cases and 183 chronically infected matched controls) and 353 European Americans (108 clearance and 245 chronic) were examined. In African Americans two linked regions, one at *IL10* and the other from *IL19/IL20*, were associated with the HCV clearance as viewed by allelic, codominant, and dominant models using conditional logistic regression ( $p=0.03-0.002$ ). We used the EM algorithm to reconstruct haplotypes within genes, and associations with clearance remained ( $p=0.05-0.01$ ). These analyses were not significant in European Americans. Our results indicate that *IL10* and *IL19/IL20* gene variants may be involved in clearance of HCV in the African American population. Funded in part by DHHS#N01-CO-12400.

**Comparative Genetic Study of Tuberculosis and Asthma Susceptibilities.** A. Poon<sup>1, 7</sup>, C. Laprise<sup>2</sup>, A. Jimnez-Corona<sup>3</sup>, M. Palacios-Martnez<sup>3</sup>, J. Sifuentes-Osornio<sup>4</sup>, A. Ponce-de-Len<sup>4</sup>, M. Bodadilla<sup>4</sup>, M. Kato<sup>4</sup>, M. Lemire<sup>5</sup>, A. Montpetit<sup>5</sup>, Y. Renaud<sup>5</sup>, P.M. Small<sup>6</sup>, M.L. Garca Garca<sup>3</sup>, T.J. Hudson<sup>5, 7</sup>, E. Schurr<sup>7</sup>. 1) Dept Biochemistry, McGill University, Montreal, QC, Canada; 2) Universit du Qubec Chicoutimi, Chicoutimi, QC, Canada; 3) Instituto Nacional de Salud Pblica, Cuernavaca, Mexico; 4) Instituto Nacional de Ciencias Mdicas y de Nutricin Salvador Zubirn, Mexico City, Mexico; 5) McGill University and Genome Quebec Innovation Centre, Montreal, QC, Canada; 6) Medicine, Stanford University, CA, USA; 7) McGill Centre for the Study of Host Resistance, Montreal, QC, Canada.

Epidemiologic evidence suggests that on the population level tuberculosis (TB) is protective for asthma/atopy. Consequently, genetic variants predisposing to TB may have been selected due to their protective effect for asthma susceptibility. We tested this hypothesis (i) by analysing bi-allelic genetic variants in four selected candidate TB susceptibility genes for association with asthma/atopy and (ii) by studying a hypothesized inverse association of asthma associated polymorphism with TB in a TB case control group. We analyzed gene-specific panels of 37 single nucleotide polymorphisms (SNPs) for *NRAMP1* (4 SNPs), *TNFA* (1 SNPs), *VDR* (19 SNPs) and *CARD15* (13 SNPs) in a panel of 421 nuclear asthma families. Haplotype structures for the asthma families were established for *NRAMP1*, *VDR* and *CARD15* and a clear linkage disequilibrium block structure was found across all three genes. We then selected several SNPs within these genes for further analysis in a group of TB cases and controls: No significant association with TB was observed for *NRAMP1*, *CARD15* and *TNFA* polymorphisms. However, two *VDR* markers, a *FokI* polymorphism located at the translational start site of *VDR* and a *TaqI* polymorphism located in the 3' region of the gene, showed evidence for association with TB. Specifically, homozygous carriers of the rare allele for the *TaqI* polymorphism were found associated with TB susceptibility ( $p = 0.027$ ). This polymorphism had previously been linked with altered expression levels of the *VDR* gene and is associated with altered bone mineral density.

**What causes early onset breast cancer in a population-based sample of Australian women with a strong family history?** *M.C. Southey<sup>1</sup>, A.A. Tesoriero<sup>1</sup>, S.G. Royce<sup>1</sup>, L. Smith<sup>1</sup>, P. MacAulay<sup>1</sup>, J. Cui<sup>1</sup>, M.A. Jenkins<sup>1</sup>, G.S. Dite<sup>1</sup>, R.L. Milne<sup>1</sup>, G. Chenevix-Trench<sup>2</sup>, A. Spurdle<sup>2</sup>, G.G. Giles<sup>4</sup>, M.R.E. McCredie<sup>3</sup>, D.J. Venter<sup>1</sup>, J.L. Hopper<sup>1</sup>, Australian Breast Cancer Family Study.* 1) University of Melbourne, Melbourne, Victoria, Australia; 2) Queensland Institute of Medical Research, Brisbane, Australia; 3) The University of Otago, New Zealand; 4) The Cancer Council, Victoria, Australia.

The Australian Breast Cancer Family Study (ABCFS) includes a population-based case-control-family study of 856 women diagnosed with breast cancer before the age of 40 years. Eight percent of cases had a strong family history, defined by having two or more first- or second-degree relatives on the same side of the family with breast or ovarian cancer, compared with one percent of controls. Of these 66 cases with a strong family history: 8 (11%) would be expected by chance alone based on the age distribution of the relatives and the population incidence rates; 14 (20%) were found to carry a germline mutation in the coding or splice site regions that were likely to be pathogenic by sequencing the coding and exon-flanking intronic regions of BRCA1 and BRCA2; 3 (4%) carry a large deletion in BRCA1; 1 (1%) is known to be part of a Li-Fraumeni kindred and carries a germline p53 mutation; 2 (3%) carry specific mutations in ATM (IVS10-6 T>G and T7271G); and 1 (1%) carries a mutation in CHK2 (1100delC). Segregation analyses of the ABCFS families have suggested that there may be as yet undetected mutations associated with a high dominantly-inherited risk. Two-loci segregation analyses also suggest that there may be genetic loci involved with a very high recessively-inherited risk. These segregation analyses cannot discount that there may also be a polygenic background of multiple genes with small effects. In summary, more than two-thirds of these population-based multiple-case breast cancer families are not explained by mutations in BRCA1, BRCA2, or other known or suspected susceptibility genes, and most of these unexplained families may be explained by recessive or polygenetic inheritance, rather than dominant inheritance or chance.

**CCR5 Pathway candidate genes susceptible to HIV-infection.** *S. Shrestha*<sup>1,2,5</sup>, *S.A. Strathdee*<sup>2</sup>, *N. Galai*<sup>2</sup>, *D. Thomas*<sup>3</sup>, *D. Vlahov*<sup>4</sup>, *S.J. O'Brien*<sup>5</sup>, *M.W. Smith*<sup>1,5</sup>. 1) Basic Research Program, SAIC, NCI-Frederick, MD; 2) Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD; 3) Division of Infectious Diseases, School of Medicine, Johns Hopkins University, Baltimore MD; 4) Center for Urban Epidemiologic Studies, The New York Academy of Medicine, New York, NY; 5) Laboratory of Genomic Diversity, NCI-NIH, Frederick, MD.

We are conducting a nested case control study within the ALIVE cohort in Baltimore to study candidate genes (CCR5, Rantes, MIP-1a, MIP-1b, MCP-2, IL10, IFNG, MCSF and IL2) involved in the CCR5 pathway of HIV infection. HIV-1 strains responsible for infection predominantly use CCR5 as a co-receptor. Homozygosity for a 32 bp deletion of CCR5 encodes a non-functional receptor preventing viral entry. However, the affect is not absolute and the allele frequency is 11% among European descent population but rare (<3%) among African Americans and absent in native Africans and Asians. We are examining other genes that up-regulate, down-regulate or bind as ligands affecting the CCR5 expression and host susceptibility to HIV infection among African American cohort with measured risk behavior. A total of 299 African American HIV-seroconverter cases and two controls per case matched for duration in the cohort using incidence density sampling are being examined for these genes. A set of informative SNPs at IL2, IL10 and IFNG have been identified and genotyped using the Taqman protocol. Single-locus association was conducted directly with cSNPs, and indirectly with other SNPs as linkage disequilibrium surrogates for functional polymorphisms. A multi-locus analysis is being conducted to test the associations between haplotypes, HIV-infection and cumulative risk exposure based on questionnaire data using conditional logistic regression. Haplotypes for informative SNP combinations at each gene are estimated via an Expectation-Maximization algorithm. Preliminary univariate analyses indicate significant ( $p < 0.05$ ) genotype association at a few loci in all three genes and also significant haplotype association in IL2, but need further analysis. Funded in part by DHHS#N01-CO-12400.

**Phylogenetic Analysis of Slavic Lebers Hereditary Optic Atrophy (LHON) Cases: The Role of Population Genetic Markers for mtDNA Human Pathology.** *S.I. Zhadanov<sup>1, 2</sup>, T.G. Schurr<sup>1</sup>*. 1) Dept Anthropology, University of Pennsylvania, Philadelphia, PA; 2) Institute of Cytology and Genetics, Novosibirsk, Russia.

One of the most puzzling features of the numerous common mtDNA diseases is the difference in population distribution and disease expression related to ethnic and genetic factors. It has been shown that certain polymorphic genetic markers in some mtDNA haplogroups promote the phenotypic expression of human diseases. The interaction between population-specific mtDNA variation and pathogenic mutations is particularly evident in LHON. Numerous studies have demonstrated that LHON cases occur in patients with haplogroup J mtDNAs six to eight times more frequently than expected based on normal control haplogroup frequencies and a random pattern of pathogenic mutation distribution in Caucasian populations. However, much less is known about such patterns in Slavic populations. For this reason, we analyzed the mtDNA genetic population background for about 30 clinical LHON cases in persons of Slavic origin. The majority of these cases occurred in individuals having common West Eurasian haplogroups (HV, TJ, UK, XI), while a small portion occurred in Eastern Eurasian haplogroup D, which is nearly absent in Slavs. One-fourth of the LHON cases (~25%) fell within haplogroup TJ, a pattern consistent with that previously seen for Caucasian populations. In addition, we observed a number of LHON cases without known mtDNA mutations, with these being distributed more randomly across mitochondrial backgrounds. When the distribution of primary LHON mutations was considered, haplogroup JT was associated with these mutations over three times higher (40%) than with cases caused by unknown mutations. Such striking geographic and population differences suggest that ancient genetic backgrounds could affect the expression of different LHON mutations. At the same time, they may also indicate that specific population-specific mtDNAs are functionally different and, hence, might have been influenced by selection. The probable interaction of the genetic background with pathogenic mtDNA mutations further supports a polygenetic model for OXPHOS disease development.

**Complex Inheritance and Possible Imprinting in Juvenile Myoclonic Epilepsy.** *D. Pal<sup>1</sup>, M. Durner<sup>1,2</sup>, I. Klotz<sup>2</sup>, E. Dicker<sup>2</sup>, S. Shinnar<sup>3</sup>, S. Resor<sup>4</sup>, J. Cohen<sup>5</sup>, C. Harden<sup>6</sup>, S. Moshe<sup>3</sup>, H. Kang<sup>3</sup>, E. Bromfield<sup>7</sup>, D. Greenberg<sup>1,2</sup>, New York Epilepsy Study.* 1) Psychiatry, Columbia Univ, New York, NY; 2) Biostatistics, Columbia Univ, New York, NY; 3) Albert Einstein College of Medicine, NY; 4) Columbia Presbyterian Medical Center, NY; 5) Beth Israel Medical Center, NY; 6) Cornell University, NY; 7) Boston University College of Medicine.

**Background.** Juvenile Myoclonic Epilepsy (JME) is a common adolescent-onset idiopathic generalised epilepsy (IGE) with complex inheritance. A major susceptibility locus (EJM1) for JME has been localised on chromosome 6, with evidence of maternal inheritance in EJM1-linked families. EJM1 was recently identified as the BRD2 gene. We sought epidemiological support for imprinting in JME families with both positive and negative evidence for linkage at EJM1, and compared patterns of seizure inheritance between JME and other IGE families. **Methods.** We ascertained 89 families through a proband with typical JME and 50 families through a proband with a non-JME IGE. Family members were assessed for seizures and EEG abnormalities. Lineality was assessed in 43 multigenerational families. The crude prevalence of seizures, epilepsy and EEG traits in 806 family members was tabulated. **Results.** Multigenerational families showed evidence for preferential maternal transmission of JME, both in EJM1-linked and unlinked families. The sex ratio was extremely skewed in JME, with a female excess not seen in other IGEs (Risk Ratio 12.5). Proband seizure types and sex influenced the risk of seizures in relatives: i) absence seizures in JME probands increased (0.16 vs 0.07) the risk of absence and GTCS (but not myoclonic seizures) in first degree relatives; ii) overall risk of seizures was doubled in relatives of male compared to female JME probands (0.15 vs 0.07). **Conclusions.** Imprinting seems the best explanation of maternal inheritance in JME. We confirmed a striking female excess in JME which may be explained by sex-dependent trait penetrance. Absence and myoclonic seizures were inherited independently, and GTCS shared susceptibility with both absence and myoclonic seizures. Our results support an oligogenic model for adolescent-onset IGE.

**Familial aggregation of hyperemesis gravidarum.** *M. Schoenberg-Fejzo, K. MacGibbon, R. Schoenberg-Paik.*  
Hyperemesis Education and Research Foundation.

Nausea and vomiting in pregnancy, occurs in 50-90% of all pregnant women. When the symptoms are so severe that individuals require hospitalization and/or therapeutic intervention, pregnant women are diagnosed with hyperemesis gravidarum (HG). HG occurs in approximately 0.3-2.0% of pregnant women, and is characterized by severe nausea and vomiting in early pregnancy that rapidly leads to at least a 5% weight loss. Currently approximately 70% of patients are prescribed antiemetic drugs of unknown safety and efficacy to treat the symptoms of HG. In an attempt to learn more about HG, we wrote and collected surveys from over 260 affected individuals. Our study shows that the 3 most commonly prescribed antiemetics (phenergan, compazine, and tigan) are more strongly correlated with second trimester fetal demise than with having any positive therapeutic effect, suggesting a better understanding of the disease and new treatments is critical. To determine whether HG is amenable to genetic studies, we included a number of questions about family history. All patients were diagnosed by a medical professional to have hyperemesis gravidarum. Of the 260 respondents, 96 reported having at least one sister with a pregnancy history. Of the 96 sister pairs, 23 (24%) reported a sister with HG. In addition, 28 (11%) affected individuals reported having an affected mother, and 29 (11%) reported having an affected secondary relative. We have also identified 14 families with 3 or more affected individuals. This study shows the frequency of HG in families is way above the reported 0.3-2.0% in the general population. This is the first evidence that there may be a genetic component to the severe form of morning sickness, hyperemesis gravidarum, which may make it amenable to genetic studies of complex disease. We hope that this will lead to a new field of study to identify the genetic basis for hyperemesis gravidarum and lead to development of targeted therapy.

**The familial occurrence of Stuttering.** *R. Uppala<sup>1,3</sup>, D. Dasgupta<sup>1</sup>, J.V. Solanki<sup>2</sup>, R. Uppala<sup>3</sup>.* 1) Dept. of Biochemistry, Institute of Science, Mumbai; 2) Dept. of Animal Genetics & Breeding, Veterinary college, Anand, India; 3) GeneHealth, Green Cross Voluntary blood bank & Genetic Centre, Paldi, Ahmedabad,.

Stuttering is a speech disorder, which begins in childhood between the ages of 3-6 and is characterized by involuntary syllable repetitions, sound prolongations or interruptions (audible and silent). It is a painful symptom and is considered as society's hidden disability. Stuttering greatly interferes with a child's emotional and psychological development and also affects their daily social or occupational functioning. The incidence of stuttering among the general population is about 0.7-1.0% with a higher 4-5:1 ratio of male to females affected. Family, twin, and adoption studies strongly suggest a genetic predisposition/susceptibility to stuttering, but no genes have yet been identified. Associations between childhood and/or adult stuttering and brain disorders, such as Down syndrome, Parkinson's disease, Tourette syndrome and depressive illness, have been reported. We have studied nine large Indian pedigrees with stuttering (OMIM 184450) in which the anomaly segregates as an autosomal dominant trait. The onset is during early childhood. These families were identified from the old records of child psychiatrists and speech pathologists and extended with information from other family members. The pedigrees consist of 455 individuals with 105 affecteds (82 males/23 females). These include two large pedigrees with 32 and 37 affecteds. The age distribution of these affecteds is 8-70 years. Severity of the phenotype was quite variable among the families and skipping of a generation was observed in two pedigrees. In three families four members were affected with post stuttering epilepsy. No other associated anomalies were present in these families. The present study indicates a monogenic mode of inheritance with variable expression. Systematic genome-wide linkage analyses, using rare larger families that show autosomal dominant stuttering may reveal one or more chromosomal loci for the phenotype. The identification and characterization of this stuttering gene will contribute in determining molecular pathophysiological basis of this disorder Email: u\_c\_rao@hotmail.com.

**Linkage disequilibrium mapping in the Newfoundland population: a reevaluation of the refinement of the Bardet-Biedl Syndrome 1 critical interval.** *Y. Fan<sup>1</sup>, J.S. Green<sup>2</sup>, P.S. Parfrey<sup>2</sup>, W.S. Davidson<sup>1</sup>*. 1) Dept Molec Biol & Biochem, Simon Fraser Univ, Burnaby, BC, Canada; 2) Faculty of Medicine, Memorial University, St. John's, NF, Canada.

Two criteria must be met for linkage disequilibrium (LD) mapping: 1) there should be a single disease causing allele with a relatively high frequency in the population, and 2) the disease causing allele should have been introduced sufficiently long ago that recombinations have reduced the original haplotype around this allele. The island population of Newfoundland has the potential for LD studies based on the nature of its founding and its subsequent isolation. Young et al. (*Am J Hum Genet* 65: 1680-1687, 1999) used 6 families in a LD analysis to refine the critical region of Bardet-Biedl Syndrome 1 (BBS1) to 1 cM. However, when Myktyyn et al. (*Nat Genet* 31: 435-438, 2002) identified the BBS1 gene it was located outside this region. Possible reasons for this are: 1) another gene is responsible for BBS in these families, or 2) the Newfoundland population is not ideal for LD studies. We screened the 6 families for mutations in the BBS1 gene. All affected individuals in 5 of the families were homozygous for the most common BBS1 mutation, M390R. However, there was no evidence for any mutation in the coding regions or exon-intron boundaries of the BBS1 gene in the affected individual in family B12. Therefore, the first condition for an effective LD analysis was not met. It should be noted though that family B12 was not a key factor in predicting the critical region of BBS1. We then performed fine haplotype mapping on a 450 kb region surrounding the BBS1 gene. Haplotypes were constructed for 8 BBS1 patients homozygous for M390R, 31 heterozygous parents and siblings, 10 sibling non-carriers, 6 apparently unrelated individuals from the general population who were heterozygotes, and 6 non-carriers of M390R from the general public. All 53 chromosomes carrying the M390R mutation contained a common sub-haplotype defined by 5 markers. This provides evidence for a single ancestral founder for BBS1 in Newfoundland. However, the same sub-haplotype (apart from M390R) was also found in 13 of 69 normal chromosomes.

**The impact of inaccurate ages on a potential association between tuberculosis and NRAMP1. C.M.T.**

*Greenwood<sup>1,2</sup>, J. Beyene<sup>1,2</sup>, S. Malik<sup>3,4</sup>, T. Egualé<sup>3,5</sup>, A. Kifle<sup>6</sup>, A. Habte<sup>6</sup>, A. Tadesse<sup>6</sup>, I. Abraham<sup>6</sup>, S. Britton<sup>7</sup>, E. Schurr<sup>3,4</sup>.* 1) Hospital for Sick Children Research Institute, Toronto, ON, Canada; 2) Department of Public Health Sciences, University of Toronto, ON; 3) Research Institute of the McGill University Health Centre, Montreal, QC, Canada; 4) Department of Medicine, McGill University, QC; 5) Department of Epidemiology and Biostatistics, McGill University, QC; 6) Armauer Hansen Research Institute, Addis Abbaba, Ethiopia; 7) Department of Medicine, Karolinska Institute, Sweden.

Covariates can be incorporated into models testing for linkage/disequilibrium; one straightforward approach involves rewriting the standard TDT as a logistic model of transmissions versus non-transmissions for a particular allele from heterozygous parents. The dependence of any association upon factors measured in the child can then be investigated. When covariates are measured with error, it is well known that standard errors tend to be too small, and that the estimates of the covariate effects may be biased. However, measurement error is not always considered in family-based association studies. Proband with proven tuberculosis and their parents were enrolled into a study in Ethiopia investigating candidate genes for association with disease susceptibility. Four NRAMP1 markers were genotyped, and haplotypes were estimated. There were 43 heterozygous parent-child transmissions involving one particular haplotype, where all four markers were genotyped in both parents and the proband, and we identified a potential association that appeared to depend on age, so that a stronger association is apparent at younger proband ages (estimated increase in odds of transmission of the risk haplotype per 5 years younger = 2.25, significance=0.012). We were concerned that self-reported age was inaccurate, with the error increasing with the age of the individual. We therefore placed a log-normal distribution on the true age, and found that the association's dependence on age appeared slightly stronger (odds=2.50, significance=0.010), but that conclusions were not altered. The impact of risk factor measurement error should be evaluated in genetic studies.

**Odds-ratio regression models for testing joint transmission of candidate genes for tuberculosis.** *J. Beyene*<sup>1, 2</sup>, *C.M.T. Greenwood*<sup>1, 2</sup>, *S. Malik*<sup>3, 4</sup>, *T. Egualé*<sup>4, 5</sup>, *A. Kifle*<sup>6</sup>, *A. Habte*<sup>6</sup>, *A. Tadesse*<sup>6</sup>, *I. Abraham*<sup>6</sup>, *S. Britton*<sup>7</sup>, *E. Schurr*<sup>3,4</sup>. 1) Population Health Sciences, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Public Health Sciences, University of Toronto, ON; 3) Research Institute of the McGill University Health Centre, Montreal, QC, Canada; 4) Department of Medicine, McGill University, QC; 5) Department of Epidemiology and Biostatistics, McGill University, QC; 6) Armauer Hansen Research Institute, Addis Ababa, Ethiopia; 7) Department of Medicine, Karolinska Institute, Sweden.

There is growing evidence linking tuberculosis susceptibility with host genetics and a number of candidate genes have been identified. For the case-parental control design, marginal transmission rates for individual marker alleles can be investigated using standard regression models. However, in genetic studies joint association parameters are of interest and models that allow explicit specification of the association parameters and link them with covariates are useful. Data have been collected in Ethiopia from probands with proven tuberculosis and their parents to investigate candidate genes for association with disease susceptibility. We studied the joint association of marker alleles for one marker at surfactant protein gene 1 and four NRAMP1 markers. The generalized estimating equations (GEE) approach was applied to test for association using the odds ratio of joint allelic transmission as the parameter of interest. We found a marginal association between the surfactant marker and one of the NRAMP1 markers ( $p = 0.0539$ ). Further modeling of the association parameter stratified by gender showed a significant interaction between the two genes in females ( $p = 0.0365$ ). Such methods can be used to investigate interactions between genes and environmental factors.

**Efficacy of haplotype-based association approaches.** *J. Drake*<sup>1</sup>, *M. Loomer*<sup>2</sup>, *C. Newton-Cheh*<sup>2,3</sup>, *A. Lochner*<sup>2</sup>, *R. Levy*<sup>2</sup>, *H. Parise*<sup>3</sup>, *S.F. Schaffner*<sup>2</sup>, *S. Gabriel*<sup>2</sup>, *M. Larson*<sup>3,4</sup>, *E.J. Benjamin*<sup>3,4</sup>, *M.J. Daly*<sup>2</sup>, *C.J. O'Donnell*<sup>3</sup>, *J.N. Hirschhorn*<sup>1,2</sup>. 1) Genetics, Children's Hosp/Harvard Med, Boston, MA; 2) Whitehead/MIT Center for Genome Research, Cambridge, MA; 3) NHLBI.Framingham Heart Study, Framingham, MA; 4) Medicine, Boston University, Boston, MA.

The genome contains blocks of linkage disequilibrium (LD), within which most chromosomes carry one of a few common haplotypes (e.g. Gabriel et al. 2002). Single nucleotide polymorphisms (SNPs) that tag these haplotypes (htSNPs) should thus capture information about other common variants in blocks, and enable efficient association studies. We now directly test this approach in a large dataset.

We studied 29 genes (covering 1.1 Mb) using a set of 1094 SNPs ("set 1") from dbSNP (TSC and BAC overlap SNPs only) and Celera, genotyped in 96 European-derived chromosomes from CEPH pedigrees. All genes are candidates for cardiovascular traits. We compared 3 different algorithms for defining blocks of LD, and selected htSNPs using 3 different approaches; results below are from one of these algorithms and approaches.

Within blocks, there were on average 4 common (>5%) haplotypes. To test how well the htSNPs captured unmeasured common variation within blocks, we identified 156 additional SNPs ("set 2") discovered by resequencing of these genes. 120 of the 156 "set 2" SNPs (77%) fell in the blocks defined using "set 1" SNPs. The "set 1" htSNPs largely captured these 120 SNPs: 79% of the "set 2" SNPs that fell in blocks were correlated with at least one htSNP in the block or a neighboring block ( $r^2 > 0.5$ ). Consideration of haplotypes of htSNPs further strengthened this correlation; only 15% of the 120 SNPs created a new common haplotype.

We conclude that htSNPs from existing databases can efficiently capture most common variation within blocks (in this non-African-derived population). We are using this approach to test these genes for association with cardiovascular traits, and have identified preliminary associations (see [www.cardiogenomics.org](http://www.cardiogenomics.org)).

Supported by the NHLBI Programs for Genomic Applications 5U01 HL66582.

**Choosing subsets of SNP for genotyping in association studies: htSNP and ldSNP.** *R. Lazarus, E. Silverman, B. Raby, D. Kwiatkowski, S.T. Weiss.* Channing Laboratory, Boston, MA.

Genotyping costs nearly always limit the design of large, comprehensive disease association studies. Subsets of SNPs must be chosen, in the hope that linkage disequilibrium (LD) between genotyped SNPs and any causal SNPs not genotyped will permit an association to be demonstrated. Although no provably optimal method for selecting subsets of SNP for any given genotyping budget and set of samples is available, we illustrate two potential approaches: a haplotype-tagging SNP (htSNP) method, and a novel method based on capturing pairwise LD in subsets of SNP (ldSNP).

Minimal htSNP sets were identified using BEST in haplotypes inferred with SNPHAP for SNP  $\geq 10\%$  frequency (mean 10%SNP/gene=25.8, SD=22.8) in complete resequencing data for 23 European American subjects from 137 inflammatory and innate immunity genes. For haplotypes observed twice or more, haplotype diversity was captured with no information loss in relatively small subsets (mean htSNP/gene=5.1, SD=2.1). However, htSNPs may not be ideal for analyses which rely on LD to detect causal variations not genotyped, since the best LD with one or more 10% SNP not in the set was below 0.5 in 78 genes (worst case  $r^2$  mean=0.50, SD=0.32).

LD can be captured in sets of SNPs chosen to provide a user specified minimum LD level between at least one of the ldSNPs and each of the SNPs not in the set. An algorithm which selects the smallest sets of ldSNP satisfying a pre-specified worst case LD level is described. Applying this method to the same resequencing data 10% SNPs, a minimum  $r^2$  of 0.5 required a mean ldSNP set size of 8.6 (SD=7.2). A minimum  $r^2$  of 0.8 required a mean ldSNP set size of 11.8 (SD=10.2). Useful levels of minimum LD require larger ldSNP sets and thus increased genotyping costs, compared with the htSNP approach. A hybrid approach where htSNP sets are augmented to give prespecified minimum LD levels is described.

The benefit of a haplotype-based approach combined with a prespecified minimum level of LD at analysis may make the additional genotyping costs justifiable in some study design situations.

**Patterns of linkage disequilibrium in calpain-10.** *M.G. Hayes*<sup>1</sup>, *T. Tsuchiya*<sup>2</sup>, *L. del Bosque-Plata*<sup>2</sup>, *N.J. Cox*<sup>1,3</sup>. 1) Human Genetics, Univ Chicago, Chicago, IL; 2) Biochemistry and Molecular Biology, Univ Chicago, Chicago, IL; 3) Medicine, Univ Chicago, Chicago, IL.

The q-terminus of chromosome 2 achieves a genome wide level of significance for linkage to type 2 diabetes in Mexican-Americans, and subsequent positional cloning analyses yield calpain-10 (*CAPN10*) as the candidate gene within the region (*NIDDM1*). A combination of two different three-polymorphism (SNP43, INDEL19, SNP63) haplotypes has the highest risk in Mexican-Americans, although replication studies produce confounding results among several other populations examined. This suggests *CAPN10* (or at least 43-19-63) might not be the true disease-associated gene, and that these polymorphisms may simply be in strong linkage disequilibrium (LD) with the nearby true disease-associated locus. To better understand the complex association of type 2 diabetes to polymorphisms in this region, we calculated pairwise measures of LD ( $D'$  and  $r^2$ ) for 64 markers spanning 800kb in 110 patients and 112 randomly chosen controls from the Starr County Mexican-American study. Two adjacent LD blocks spanning 120kb were discernable, one containing *CAPN10*, and the other containing a neighboring gene, *GPR35*. Pairwise LD values within *CAPN10* are significantly greater than the LD values between any marker within *CAPN10* and any marker outside of *CAPN10*. Hence, LD decays rapidly outside *CAPN10*, and suggests that it is the true diabetes-susceptibility gene and not simply a marker for a closely linked unidentified diabetes-susceptibility locus. On average, patients have higher LD values than controls. This difference was examined further by comparing mean patient and control pairwise LD values within overlapping sliding windows (4.5kb in length and sliding 1.5kb/iteration), and mean patient and control pairwise LD values between the sliding window and the remainder of the region. Both comparisons yield a significant peak in the LD difference between patients and controls in the largest intron (13<sup>th</sup> of 15) of *CAPN10*. This peak is largely the result of decreased LD among the controls, not an increase in the LD among patients.

**Patterns of genetic variation and linkage disequilibrium of genes in the renal-dopaminergic system. K.E.**

*Lohmueller<sup>1</sup>, M.H. Liang<sup>1</sup>, H. Boudreau<sup>1</sup>, L.L. Liu<sup>1</sup>, E. Wilson<sup>1</sup>, R.A. Felder<sup>2</sup>, P.A. Jose<sup>3</sup>, S.M. Williams<sup>4</sup>, L.J.C.*

*Wong<sup>1</sup>.* 1) Inst Molec & Human Genetics, Georgetown Univ Med Ctr, Washington, DC; 2) Dept of Pathology, Univ of Virginia Health Sciences Ctr, Charlottesville, VA; 3) Dept of Pediatrics, GUMC, Washington, DC; 4) Div of Cardiovascular Med & Program in Human Genetics, Vanderbilt Univ School of Med, Nashville, TN.

Association studies using single nucleotide polymorphisms (SNPs) have the potential to help unravel the genetic basis of hypertension. Nevertheless, association studies of hypertension have yielded ambiguous results thus far. Consequently, it is becoming clear that these studies must be interpreted within the context of the genetic structure of the populations studied. With this in mind, we analyzed genetic variation in the G protein-coupled receptor kinase 4 (*GRK4*) gene whose product has recently been shown to inhibit the dopamine receptor D1 (*DRD1*) from increasing sodium excretion. We genotyped the three previously identified *GRK4* SNPs (G4448T, C679T and C1711T) as well as SNPs in *DRD1* (A-48G) and *DRD5* (G181A) in the following populations: African Americans, Asians, Hispanics and Caucasians. The SNPs in *GRK4* involve amino acid changes R65L, A142V and A468V, respectively. Levels of linkage disequilibrium (LD) between the *GRK4* SNPs varied among populations, with African Americans having lower levels of LD within the gene compared to levels in other populations. However, useful levels of LD ( $r^2 > 0.33$ ) extended over 15.5 kb in all populations studied and as far as 48.6 kb in Hispanics and Caucasians. While we noted limited haplotype diversity in the four populations studied, allele and haplotype frequencies differed among ethnic groups. Though preliminary, our results not only document different allele frequencies among populations, but differences in haplotype structure that may be important in evaluating association studies with hypertension and *GRK4*, *DRD1* and *DRD5*.

**The effect of natural selection on the pattern of linkage disequilibrium around the hemoglobin E variant. J.**

*Ohashi*<sup>1, 2</sup>, *I. Naka*<sup>1</sup>, *J. Patarapotikul*<sup>3</sup>, *H. Hananantachai*<sup>3</sup>, *S. Looareesuwan*<sup>3</sup>, *A.G. Clark*<sup>2</sup>, *K. Tokunaga*<sup>1</sup>. 1) Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; 2) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 3) Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

The carriers of the hemoglobin E variant (HbE; 26Glu->Lys) are considered to confer some protection against *Plasmodium falciparum* malaria. We analyzed biallelic markers surrounding the HbE variant in a Thai population to examine the effect of natural selection on the pattern of linkage disequilibrium (LD) and to infer the evolutionary history of the HbE variant. The pairwise LD analysis of 44 markers revealed that LD between HbE and markers distal to HbE extended beyond 100 kb, whereas no LD was observed between non-HbE variants and the distal markers. The inferred haplotype network suggested that HbE had a single origin. Forward-in-time computer simulations under a variety of selection models indicated that the HbE variant arose within the past 1,240 to 4,440 years. Our results support the conjecture that a mutation of HbE occurred recently, and the allele frequency has increased rapidly due to positive selection in malarial endemic areas of Southeast Asia.

**Maternal cigarette smoking and variants in GSTP1 and GSTT1 contribute to the etiology of orofacial clefting.** *M. Shi*<sup>1</sup>, *K. Christensen*<sup>2</sup>, *A. Lozada*<sup>3</sup>, *P. Romitti*<sup>4</sup>, *J. Murray*<sup>1,3,4</sup>. 1) Dept Biological Sci, Univ Iowa, Iowa City, IA; 2) Center for the Prevention of Congenital Malformations, Institute of Public Health, Univ Southern Denmark, 5000 Odense C, Denmark; 3) Dept Pediatrics, Univ Iowa, Iowa City, IA; 4) Dept Epidemiology, Univ Iowa, Iowa City, IA.

Orofacial Clefts (OCs) have a complex etiology involving both genetic and environmental factors. Maternal tobacco smoking has been associated with OCs in some studies but unconfirmed in others. These disparities may be due to the failure to consider inherited pharmacogenetic susceptibilities to tobacco smoke. In this study, maternal cigarette smoking and the effects of variants in genes involved in the detoxification pathway for smoke byproducts were evaluated in a collection of cases and controls of OCs. Variants in genes involved in both phase I and phase II detoxification were investigated. Nine SNPs in eight genes were genotyped on samples from a Danish population and an Iowa population. A total of 370 case and 360 control case-parent triad families, and an additional 300 case and 500 control individual samples were investigated in this study. Effects of the maternal and fetal genotypes as well as the interaction between genotypes and smoking status were tested. Logistic regression and log-linear modeling were applied in the analysis. In Danish samples, even though no significant associations were detected either for the maternal or fetal genotype alone, a significant effect of the interaction between GSTP1 A313G and maternal cigarette smoking was identified in the case-parent samples. In the case-control samples, interaction between the GSTT1 null mutation and maternal smoking was identified to have a significant effect on OCs. Genotyping has been completed on the Iowa samples and replication tests are underway. In summary, significant effects on OCs were detected in the interactions between the polymorphisms in two genes of the GST family, GSTT1 and GSTP1, and maternal cigarette smoking. These results suggest that there may be individuals at particular high risk for abnormal pregnancy outcomes in the context of maternal smoking and may be a target group for especially aggressive antismoking programs.

**Association of a DBH Intron 5 Taq I Polymorphism and Plasma Dopamine beta-hydroxylase Activity Results from Linkage Disequilibrium to a Putative Functional Variant, -1021C->T.** *Y. Tang<sup>1</sup>, S. Buxbaum<sup>2</sup>, M. Kohnke<sup>3</sup>, G. Anderson<sup>4</sup>, J. Cubells<sup>1</sup>, Lab of Human Genetics.* 1) Department of Psychiatry, Yale University School of Medicine VA Connecticut Health Care System, Psychiatry/116A2, 950 Campbell Avenue, West Haven, CT 06516; 2) Department of Human Genetics, University of Pittsburg, Pittsburg, PA; 3) University Hospital of Psychiatry and Psychotherapy, Tübingen, Germany; 4) Yale Child Study Center, Yale University, New Haven, CT.

Background: Plasma activity of dopamine beta-hydroxylase (DbetaH), the enzyme facilitating the conversion of dopamine to norepinephrine, is an important endophenotype. Plasma DbetaH is under genetic control by the structural locus encoding DbetaH protein, DBH. A common polymorphism, IVS5+192CT, which is located in exon 5 and is easily genotyped by restriction with Taq I, has been reported to associate with attention deficit hyperactivity disorder. Its relationship to functional variation at DBH is therefore of keen interest. This study tested the following hypotheses: (1) IVS5+192C->T associates with plasma DbetaH level; and (2) there is linkage disequilibrium between IVS5+192C->T and a putative functional polymorphisms, -1021C->T, located in the proximal 5 region of the DBH gene. Methods: Plasma DbetaH activity was measured in a mixed sample consisted of cocaine abusers, alcoholics, patients with affected disorders and normal controls (n=447). Genotypes were determined at Taq I and -1021c->T functional DBH polymorphisms. Results: (1) The mean square-root plasma DbetaH levels were 6.14+/-1.70, 5.72+/-1.81 and 4.40+/-2.07 nmol/min/ml for CC (n = 68), CT (n=223) and TT (n=156) groups respectively (F=29.692, p<0.000); (2) There was strong linkage disequilibrium between IVS5+192C->T and -1021C->T polymorphisms. (3) Multivariate analysis showed that IVS5+192C->T genotype did not explain significant additional variance in plasma DbetaH levels, beyond the effect of 1021C->T. Conclusions: IVS5+192C->T is associated with plasma DbetaH activity, with the T allele associating with lower plasma DbetaH activity. This association reflects linkage disequilibrium between IVS5+192CT and 1021C->T.

**Patterns of gametic disequilibrium between microsatellite loci on human chromosomes Xq and 11p.** *E. Sande<sup>1</sup>, J. Hermida<sup>1</sup>, L. Rodríguez<sup>1</sup>, S. Rodríguez<sup>1</sup>, C. Martín<sup>2</sup>, C. Zapata<sup>1</sup>.* 1) Departamento de Genética, Universidad de Santiago, 15782 Santiago de Compostela, Spain; 2) Departamento de Dermatología y Otorrinolaringología, Universidad de Santiago, 15782 Santiago de Compostela, Spain.

Knowledge on distribution of nonrandom associations between alleles at different loci (gametic disequilibrium) along human genome is essential for understanding its multilocus architecture as well as for designing and interpreting an experience of localization of disease genes. Here, we report a comparative study of levels of disequilibrium between microsatellite loci located spread across extensive anonymous regions on human chromosomes Xq and 11p, from a large sample of the Galician population (northwest Spain). We found that significant disequilibrium between microsatellite pairs is substantially higher along Xq and 11p than that previously reported for different human chromosomes in populations with similar demographic history, although the proportion of interallelic non-random associations was smallest over chromosome X. Moreover, patterns of disequilibrium depend on allele frequencies and their sizes. In particular, rare alleles (frequency 3%) and alleles of extreme size tend to exhibit greater disequilibrium than the other alleles. Distribution of disequilibrium along these extensive regions of human chromosomes seems to be complex and it does not follow simple rules depending on between-locus recombination frequency. In fact, significant correlation between the intensity of GD and recombination frequency and physical distance was not detected. Our observations suggest that the high rate of mutation of microsatellite loci together with their complex mutational dynamics are probably the main causal factors contributing to the origin and distribution of disequilibrium detected across Xq and 11p. Consequently, patterns of disequilibrium between microsatellites on human chromosomes seems to be rather unspecific of chromosome or chromosome region.

**A common founder mutation in *FKRP* causes limb girdle muscular dystrophy type 2I (LGMD2I) in both Hutterite and European populations.** P. Frosk<sup>1</sup>, C.R. Greenberg<sup>1, 2</sup>, A. Poulin<sup>1</sup>, R. Lamont<sup>1</sup>, E. Nylen<sup>1</sup>, M. Zaik<sup>3</sup>, V. Straub<sup>3</sup>, K. Bushby<sup>4</sup>, D. Frappier<sup>5</sup>, N.M. Roslin<sup>5</sup>, K. Morgan<sup>5, 6</sup>, T.M. Fujiwara<sup>5, 6</sup>, K. Wrogemann<sup>1, 2</sup>. 1) Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, Canada; 2) Department of Pediatrics, University of Manitoba, Winnipeg, Canada; 3) Department of General Pediatrics and Neuropediatrics, University of Essen, Essen, Germany; 4) Institute of Human Genetics, University of Newcastle upon Tyne, Newcastle upon Tyne, UK; 5) Departments of Human Genetics and Medicine, McGill University, Montreal, Canada; 6) The Research Institute of the McGill University Health Centre, McGill University, Montreal, Canada.

The limb girdle muscular dystrophies (LGMDs) are a clinically and genetically heterogeneous group of disorders characterized by weakness and wasting in the pelvic and shoulder girdles. They are relatively rare worldwide, ranging from 1/14,500 to 1/123,500. They are common in the Hutterite population of North America with a prevalence of approximately 1/500. Using samples from Hutterite families, we have identified a putative disease-causing mutation in each of two autosomal recessive LGMDs: a *TRIM32* missense mutation (c.1459G>A, D487N) in LGMD2H and a *FKRP* missense mutation (c.876C>A; L276I) in LGMD2I. The LGMD2H mutation appears to be private to the Hutterite population and no other *TRIM32* mutations have been reported. In contrast, the LGMD2I mutation may be the most frequent LGMD-causing mutation in European patients. A single common haplotype surrounding the *FKRP* gene was identified in the Hutterite LGMD2I patients. Thirteen non-Hutterite LGMD2I patients of northern European origin share a core 2-marker haplotype of 0.19 Mb with the Hutterite haplotype. The occurrence of the L276I mutation on a common core haplotype suggests that it is a founder mutation which is widely dispersed in populations of European ancestry.

**Selecting a minimal set of SNPs for whole-genome association studies.** *M.A. Eberle<sup>1</sup>, C.S. Carlson<sup>2</sup>, D.C. Crawford<sup>2</sup>, M.J. Rieder<sup>2</sup>, D.A. Nickerson<sup>2</sup>, L. Kruglyak<sup>1</sup>.* 1) Department of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Department of Genome Sciences, University of Washington, Seattle, WA.

Roughly 6 million sites in the human genome are polymorphic in the human population with frequency of both alleles above 5-10%. These common SNPs include the genetic variants proposed to be involved in disease susceptibility under the common disease-common variant hypothesis. The most straight-forward approach to test for associations between genetic variants and common diseases would be to compare the frequencies of all 6 million common SNPs in a large case-control study. Because this is not feasible with current or near-future technologies (at least not at a reasonable cost), a key goal of human genetics is defining a subset of SNPs that effectively assays the entire set through linkage disequilibrium (LD) between those SNPs included in the subset and the rest. Two central issues of this goal are: (1) finding the minimal number of SNPs that must be included in the subset in order to preserve high power to detect phenotypic association with any SNP and (2) deciding how to most effectively select this subset from the entire set of 6 million SNPs. We have examined these issues by performing coalescent simulations using population parameters that match empirically observed patterns of LD. For each series of population simulations we compare the power to detect association between common genetic variants and disease with subsets of SNPs chosen on the basis of random selection, pairwise LD, allele frequency and haplotype blocks. Initial results indicate that while a minimal subset of ~200,000-500,000 SNPs will have 80-90% power to detect associations realistic selection strategies will require larger numbers of SNPs.

**Oligogenic segregation analysis of obesity-related traits in hypertensive families from a French-Canadian isolate.**

*F. Gagnon<sup>1</sup>, J. Freil<sup>1</sup>, D. Gaudet<sup>2</sup>, J. Tremblay<sup>2</sup>, A.W. Cowley<sup>3</sup>, Z. Pausova<sup>2</sup>, P. Hamet<sup>2</sup>.* 1) Epidemiology and Community Med, University of Ottawa, Ottawa, Canada; 2) Centre de recherche du CHUM, Montreal, Canada; 3) Medical College of Wisconsin, Milwaukee, USA.

Obesity (Ob) is strongly related to morbidity and mortality and is drastically rising in North America. Ob is a complex disorder with multiple genes likely to be involved. Several genome scans of Body Mass Index have been performed but linkage results are inconsistent across studies. Little work has been done to identify better Ob-related quantitative traits (QT) prior linkage analysis. Physiological and statistical relationships between Ob and blood pressure are well established, and genetic pleiotropic effects on these traits have been suggested. In this study, we used 97 hypertensive families (n=1180) from a relative isolate population with the purpose of identifying the most promising QT for genome scan analyses of Ob-related traits in hypertension (HT). More than 600 phenotypes have been collected. Since body water regulation has been suggested to be involved in Ob-related HT, 2 body water measures as estimated by bioimpedance, in addition to 5 anthropometric measures, have been analyzed in n=242-792 individuals. Using novel oligogenic segregation analysis based on Bayesian Markov chain Monte Carlo methods, our analyses provide evidence in support of at least 1 locus with an individual contribution > 10 % to each trait variance. Contributions of the largest loci to the variance of anthropometric QT are ~ 41-64 %, and ~ 11-30 % for body water QT. Among the QT studied, subscapular skinfold is the best QT candidate because of the small number of loci implicated (mean 3.9), with the 3 largest loci contributing ~55, 20 and 7 %, respectively, to the variance. Age and sex were included as covariates in all QT analyses. A strong sex effect (21-42 %) on the variance was estimated for body water QT. Careful analyses of QT related to weight and water regulation prior linkage analyses will be useful in mapping genes implicated in Ob-related hypertension. Analysis of more Ob-related QT is under way.

**A cost-effective statistical and experimental approach to the analysis of candidate genes in complex disease using haplotype tag SNPs.** C.E. Lowe, J. Cooper, B.J. Barratt, E.A. Green, D. Clayton, J.A. Todd. JDRF/WT Diabetes and Inflammation Laboratory, CIMR, University of Cambridge, UK.

Owing to the high cost of single nucleotide polymorphism (SNP) typing, the search for common variants that may be expected to underlie common complex traits is currently restricted to candidate genes selected on the basis of function and data from linkage studies and animal models. Nevertheless, there may be hundreds of candidates. We have attempted to optimise the interrogation of any single gene in order to reduce costs while retaining as much statistical power as possible. To investigate whether the *RANK* or *TRANCE* immune response genes are associated with T1D we sequenced the exons and 3 kb 5' and 3' of both genes in 48 parents of diabetics and 48 controls. In *RANK*, 24 SNPs were identified, of which 16 were common (frequency >3%). In *TRANCE*, 26 polymorphisms were identified, 12 of which were common. From these common polymorphisms we determined an optimal set of haplotype tag SNPs (htSNPs), which captured the allelic variation of the untyped SNPs with a minimum  $r^2$  of 0.8. Six and four htSNPs were chosen for *RANK* and *TRANCE*, respectively. We then employed a two-stage procedure for genotyping. Phase 1 involved genotyping 666 multiplex families and testing for association using a multi-locus transmission/disequilibrium test (mlTDT). The mlTDT *P*-values for *TRANCE* and *RANK* were 0.295 and 0.112, respectively. For *RANK* we proceeded to phase 2: genotyping htSNPs in 1,695 additional T1D families. Phase 2 analyses were then performed on the entire dataset (Phase 1 and Phase 2 data combined) for which the mlTDT *P*-value was 0.286. Power calculations indicated that there was little loss in power in this two-stage process compared to a single stage approach, although it vastly reduces the genotyping requirement. We conclude there is little evidence in these data for association between the common variants identified in these genes and T1D. However, the combination of htSNPs and a two-stage design results in an effective and efficient framework for candidate gene studies. In this study, for example, the required genotyping was reduced by approximately 75%.

**Mapping multiple sclerosis (MS) susceptibility to the HLA-DR locus in African-Americans.** *J.R. Oksenberg<sup>1</sup>, L.F. Barcellos<sup>1</sup>, B.A.C. Cree<sup>1</sup>, T.L. Bugawan<sup>2</sup>, O. Khan<sup>3</sup>, E. Mignot<sup>4</sup>, L. Lin<sup>4</sup>, S. Schmidt<sup>5</sup>, G. Thomson<sup>6</sup>, D. Reich<sup>7</sup>, M.A. Pericak-Vance<sup>5</sup>, J.L. Haines<sup>8</sup>, S.L. Hauser<sup>1</sup>, MS Genetics Group.* 1) Dept of Neurology, UC San Francisco; 2) Roche Molecular Systems, Inc., Alameda, CA; 3) Wayne State University School of Medicine, Detroit, MI; 4) Stanford University, CA; 5) Duke University Med Center, Durham, NC; 6) UC Berkeley, Berkeley, CA; 7) Whitehead Institute/MIT Center for Genome Research; 8) Vanderbilt University, Nashville, TN.

An association with the HLA-DRB1\*1501-DQB1\*0602 (DR2) haplotype in MS has been repeatedly demonstrated in high-risk (Northern European or NE) groups. However, further localization within this haplotype has been difficult since it is unknown whether the effect is explained by DRB1 or DQB1, which are in strong linkage disequilibrium (LD). Compared to NE, Africans are characterized by greater HLA haplotypic diversity and distinct patterns of LD. To localize the HLA MS gene, we analyzed DRB1 and DQB1 alleles in a large dataset of self-reported African-American MS cases (n=336), family members (n=357) and controls (n=310). Using informative SNP markers, an average of 19.1% European ancestry was present in both cases and controls. Global testing for DRB1 and DQB1 in cases and controls revealed significant differences ( $p=4.0 \times 10^{-4}$  and  $p=0.02$ , respectively). While the DQB1 effect was restricted to DQB1\*0602 only, associations with DRB1\*15 ( $p=6.0 \times 10^{-4}$ ) and DRB1\*0301 ( $p=0.01$ ) alleles were identified. As observed in NE datasets, an increased risk for DRB1\*1501 ( $p=0.03$ ) and the DR2 haplotype ( $p=0.049$ ) were both present. In addition, DRB1\*1503 was also increased in patients ( $p=0.015$ ). Remarkably, the DRB1\*1501 and 1503 associations were independent of DQB1\*0602, as chromosomes carrying this allele with other DRB1 alleles were present in cases and controls at identical frequencies (4.0% each,  $p=0.77$ ), and conversely, DRB1\*15 haplotypes without DQB1\*0602 were increased in cases ( $p=0.01$ ). Family based analyses also supported a role for DRB1, but not DQB1. Our results provide strong evidence for DRB1 in MS and underscore the power of using ethnically defined datasets to identify disease genes.

**LRP5, low density lipoprotein receptor-related protein 5, is a bone-mineral-density determinant.** *T. Mizuguchi*<sup>1</sup>, *Y. Watanabe*<sup>1</sup>, *K. Tsukamoto*<sup>2</sup>, *H. Tomita*<sup>3</sup>, *N. Niikawa*<sup>1</sup>, *K. Yoshiura*<sup>1</sup>. 1) Human Genetics, Nagasaki University, Nagasaki, Japan; 2) Departments of Pharmacotherapeutics, Nagasaki University, Nagasaki, Japan; 3) Nagasaki Prefectural Medical Health Center, Nagasaki, Japan.

Osteoporosis is a multifactorial trait with low bone mineral density (BMD) determined by bone formation and resorption. We report results of association study between BMD and candidate genes: TGFB1, TGFBR2, SMAD2, SMAD3, SMAD4, INFB1, IFNAR1, FOS and LRP5. Samples included 481 general Japanese women who had a health checkup. There was no association between their genotypes and BMD values ( $p > 0.10$ ) in any of the first 8 genes. The LRP5 gene is another candidacy, because its mutations cause autosomal recessive osteoporosis-pseudoglioma syndrome (OPPG) characterized by osteoporosis and a high-bone-mass trait, and *Lrp5*<sup>-/-</sup> mice showed phenotype similar to OPPG. With 21 (7 known and 14 novel) SNPs in LRP5, we genotyped 77 of the samples to analyze linkage disequilibrium (LD) within the gene region. We chose 9 of the 21 SNPs showing minor allele frequency  $> 0.2$ , and identified a strong LD region between two SNPs, LRP5-7 and LRP5-21. Using 15 SNPs within this LD region, we constructed 4 common haplotypes ( $> 5\%$  frequency), accounting for 89 % of all chromosomes. Association study revealed that women with one or two copy (heterozygotes or homozygotes) of Haplotype 3 had significantly lower BMD than those without it ( $p < 0.03$ ). Of 4 LRP5-SNPs that located in this LD block, three gave relatively significant results. Women with C/C genotype at LRP5-9 SNP site had higher BMD value, compared to C/T and T/T women ( $p < 0.022$ ). Likewise, G/G at LRP5-20 and C/C women at LRP5-21 showed higher BMD than those with G/A or A/A ( $p < 0.038$ ) and with C/T or T/T ( $p < 0.051$ ), respectively. Comparison between diplotypes and genotypes showed that these 3 SNPs represent Haplotype 3. LRP5-9 and LRP5-21 are both cSNPs, but result in synonymous change and A1330V, respectively. Since alterations of gene function by them are unlikely, a SNP that affects LRP5 gene function may exist within or nearby the LD region.

**The MMP3 Gene and High Myopia.** *C.C. Liang<sup>1,2</sup>, H. Xu<sup>2</sup>, H.T. Chan<sup>3</sup>, M.L. Sun<sup>4</sup>, K.S. Hung<sup>4</sup>, S. Lee<sup>2</sup>, C.W. Chou<sup>1</sup>, M.J. Wu<sup>3</sup>, W.T. Huei<sup>1</sup>, J.Y. Su<sup>1</sup>, C. Gilliam<sup>2</sup>, E. Yen<sup>1</sup>, S.H. Juo<sup>2</sup>.* 1) Kaohsiung Municipal United Hospital, Kaohsiung, Taiwan; 2) Columbia University, Genome Center, New York, USA; 3) Sun Yat-Sen University, Kaohsiung, Taiwan; 4) Chang-Gung Memorial Hospital, kaohsiung, Taiwan.

Myopia is a leading cause of loss of vision, and its prevalence is increasing. Strong genetic contribution (heritability estimated from 0.6 to 0.9) towards the development of myopia has been suggested in several twin studies. However, genetic studies to map the susceptibility genes for myopia are sparse.

In experimental myopia, eye growth is accompanied by altered proteolytic activities which could serve to remodel the structural components of the scleral extracellular matrix (ECM). The MMP3 gene is involved in tissue remodeling scleral ECM. Recent gene expression studies suggested that mRNA levels and protein activities of MMP3 changed in the induced myopic eyes. The 1612 5A/6A polymorphism of the MMP3 gene has been shown to have different expression activity in cultured cells. This polymorphism has been studied in several complex diseases, but has not been tested in myopia yet.

We investigated the relationship between the 5A/6A polymorphism and myopia in the unrelated young (aged between 17 and 45) Taiwanese population. The study participants included 179 high myopes (5D or higher) and 209 controls (1 D or lower). Genotyping was performed using the fluorescent polarization method. The 5A allele frequency was 12.3% in the cases and 13.6% in the controls. The number of participants among the three genotypes 5A/5A, 5A/6A and 6A/6A was 5, 47 and 179 in the case group, and 1, 55 and 153 in the control group. The genotypic distribution was not deviated from the Hardy-Weinberg Equilibrium in either cases or controls. The chi-square test for three genotypes between cases and controls was not significant with a p value of 0.12. The 5A frequency between cases and controls was not significantly different ( $p=0.37$ ) either. These data indicated that the 5A/6A polymorphism at the MMP3 gene may not play an important role in developing myopia in the Taiwanese population.

**A straightforward approach to evaluate false positive associations in studies of gene interaction.** *C.M. Van Duijn<sup>1</sup>, J.J. Hottenga<sup>1,2</sup>, G. Roks<sup>1</sup>, B. Dermaut<sup>3</sup>, C. Van Broeckhoven<sup>3</sup>.* 1) Epidemiology & Biostatistics, Erasmus MC, Rotterdam, Netherlands; 2) Department of Medical Genetics, Leiden University Medical Center (LUMC), Leiden, The Netherlands; 3) Department of Molecular Genetics, Flanders Interuniversity Institute for Biotechnology Born-Bunge Foundation, University of Antwerp (UIA), Antwerp, Belgium.

Research of gene-gene interactions will be important in unraveling genetic risk factors involved in complex traits. However, gene interaction studies have shown to be susceptible to false positive findings as well as false negative ones. One of the reasons may be over-stratification of a limited number of cases and controls leading to a small number of subjects in the stratum of carriers of both risk alleles. In particular the number of controls who have both risk alleles is often small, even for common polymorphisms. We present a straightforward approach to reveal spurious associations due to over-stratification in controls that can be applied in gene-gene interaction studies. Basically we propose to reformulate the data and analyse cases and controls separately. In controls, the association between two unlinked genes will indicate bias in the findings of the study. From this approach it also follows that one may improve the statistical power of the study and reduce the probability of false positive findings by genotyping controls for the second gene in the limiting stratum specifically, i.e., control carriers of the risk allele of the first gene studied. This approach may be useful in large-scale epidemiological studies in which multiple genes often have been characterized. We will illustrate the approach using an example of a study of interaction of the apolipoprotein E gene and presenilin-1 gene in relation to Alzheimer's disease. In this study a false positive association was detected using this method.

**Evidence for extensive transmission distortion in the human genome.** *S. Zöllner<sup>1</sup>, X. Wen<sup>1</sup>, N.A. Hanchard<sup>2</sup>, M.A. Herbert<sup>2</sup>, C. Ober<sup>1</sup>, J. Pritchard<sup>1</sup>.* 1) Department of Human Genetics, University of Chicago, Chicago, IL. 60637, USA; 2) Department of Paediatrics, University of Oxford, JohnRadcliffe Hospital, Oxford, OX3 9DU, UK.

It is a basic principle of genetics that each chromosome is transmitted from parent to offspring with a probability that is given by Mendel's laws. However, several known biological processes lead to skewed transmission probabilities among surviving offspring and, therefore, to excess genetic sharing among relatives. Examples are in utero selection against deleterious mutations, meiotic drive, and maternal-fetal incompatibility. Although these processes affect our basic understanding of inheritance, little is known about their overall impact in humans or other mammals. In this study we examined genome screen data from 148 nuclear families, collected without reference to phenotype, in order to look for departures from Mendelian transmission proportions. Using singlepoint and multipoint linkage analysis, we detected a modest but highly significant genome-wide shift towards excess genetic sharing among siblings (average sharing of 50.52% for the autosomes;  $p=0.001$ ). Our calculations indicate that many loci with skewed transmission are required to produce a genome-wide shift of this magnitude. We have explored possible mechanisms for the transmission distortion, and find that viability selection in utero is likely to be the most important. Since transmission distortion loci are subject to strong selection, this raises interesting questions about the evolutionary forces that keep them polymorphic. Finally, our results also have implications for mapping disease genes and the genetics of fertility.

**Multivariate diplotype analyses in genetic association studies - association of Insulin Degrading Enzyme (IDE) gene with Late Onset Alzheimer's Disease (LOAD).** *K. Lau*<sup>1</sup>, *C. Rowland*<sup>1</sup>, *A. Grupe*<sup>1</sup>, *A. Goate*<sup>2</sup>, *J.C. Morris*<sup>2</sup>. 1) Celera Diagnostics, Alameda, CA; 2) Washington U., St. Louis, Missouri.

In case-control studies, comparing diplotype (pair of haplotypes in diploids) frequencies can be more powerful than comparing haplotypes, especially when the mode of inheritance (MOI) is recessive. Multivariate analyses using diplotypes, assessing joint effects of multiple loci, environmental factors and interactions, can be performed using multiple logistic regression. The main issues involve the choice of SNPs, diplotype inference and dealing with rare diplotypes. We illustrate the analysis methods using a case-control study with 419 LOAD cases and 375 controls. 3 SNPs within the IDE gene have been genotyped. APOE genotypes, gender, age of onset and years of education are important factors for LOAD. Genotypic association tests for individual markers reveal that SNPs IDE-A and IDE-B are associated in the APOE4+ stratum when a recessive MOI is used. P-values are 0.051 and 0.073 while odds ratios OR are 0.36 (0.12-1.0 95% CI) and 0.42 (0.16-1.08) for IDE-A and B, which are in strong LD ( $R^2=0.74$ ,  $D'=0.98$ ). Haplotype and diplotype analyses were performed on IDE-A & B only as the third SNP is not in LD ( $R^2<0.5$ ). No significant differences in haplotype frequencies were found using the program HAPLO.SCORE (Schaid et. al. AJHG 70:425-434, 2002). A multiple logistic regression model was built with IDE-AB diplotypes, age of onset, gender, years of education, APOE4 status and APOE4xIDE-AB interaction. Diplotypes were inferred using SNP HAP (D.Clayton's website) and weighted with the inference probabilities in analyses using SAS. Recessive MOI was used to construct design variables for IDE-AB. APOE4xIDE-AB interaction term was significant (p-val.=0.006). In stratum specific analyses, IDE diplotypes were not significantly associated in the E4- stratum. For the E4+ stratum, the p-value for the diplotype association, adjusted by other factors, is 0.019, OR=0.28 (0.099-0.81). Exact logistic regression has been performed but results do not change significantly. While diplotype analyses show significant association between IDE SNPs and LOAD, haplotype analyses do not.

**Mitochondrial DNA haplogroups and maternal transmission of type 2 diabetes.** *M.L. Langdown<sup>1</sup>, M.R. Nelson<sup>1</sup>, N.J. Markward<sup>1</sup>, C. Herrnstadt<sup>2</sup>, S. Kammerer<sup>1</sup>, T. Haak<sup>3</sup>, H. Northoff<sup>4</sup>, A. Braun<sup>1</sup>, P. Meyer<sup>5</sup>.* 1) Sequenom Inc., San Diego, CA; 2) San Diego, CA; 3) Research Institute of the Diabetes Academy, Bad Mergentheim, Germany; 4) Department of Transfusion Medicine, University Hospital of Tuebingen, Germany; 5) Institute of Human Genetics, University Hospital of Tuebingen, Germany.

Many studies have implicated that maternal transmission may influence the etiology of type 2 diabetes and that the mitochondrial genome, which is strictly maternally inherited, might play a significant role. In a collection of unrelated patients with type 2 diabetes from Germany, we observed a significant increase in the number of individuals reporting a history of maternal diabetes (40%) compared to paternal diabetes (21%). This excess was not seen in age- and gender-matched controls (10%). Thus, we tested whether the major European mitochondrial DNA (mtDNA) haplogroups are associated with disease and/or maternal history of type 2 diabetes. MtDNA was extracted (498 cases and 498 controls) and genotyped at nucleotide positions which define the nine European mtDNA haplogroups (H, I, J, K, T, U, V, W, X), the two predominant Asian haplogroups (A, B) and the African/Asian superhaplogroup (M). As expected, the most predominant mtDNA haplogroup was H, with a frequency of 40%. The Asian and African mtDNA haplogroups were of low frequency (<1%) and were excluded from further analysis. Haplogroup frequencies were compared among cases and controls, yielding no significant evidence of an association between a specific mtDNA haplogroup and type 2 diabetes. Among type 2 diabetic cases, maternal history of diabetes was significantly reduced from 40% to 20% in the IXW cluster ( $P < 0.01$ ). We found no significant difference in paternal history of diabetes. This study leads us to conclude that the maternal inheritance of type 2 diabetes may not be solely dependent upon the transmission of mtDNA. Rather, other genetic phenomena such as interaction of mitochondrial and nuclear DNA polymorphisms, maternal imprinting or intrauterine environment may predispose an individual to type 2 diabetes and contribute to the onset and severity of the disease.

**Screening of the neurofilament M Gene Gly336Ser mutation in a French-Canadian population with Parkinson's disease.** *F. Han*<sup>1</sup>, *D.E. Bulman*<sup>1,3</sup>, *M. Panisset*<sup>2</sup>, *D.A. Grimes*<sup>1,3</sup>. 1) Molecular Medicine, Ottawa Health Research Institute, Ottawa, Ontario, Canada; 2) McGill Centre for Studies in Aging, McGill University, Montreal, QC, Canada; 3) Department of Neurology, University of Ottawa, Ottawa, ON, Canada.

Neurofilament M is one of the three neurofilament subunits—the light, medium, and heavy neurofilaments (NF-L, NF-M and NF-H) which are the most abundant intermediate filaments (IFs) in neurons. Abnormalities in these proteins have been associated with neurological diseases in humans and animal models. Recently, a single base pair substitution (G1747A) mutation of the NF-M gene was reported in a French-Canadian patient with early onset Parkinson's disease (PD). This mutation resulted in the amino acid substitution Gly336Ser. Three unaffected siblings were found to be heterozygotes for the NF-M Gly336Ser mutation but to date no other affected PD individuals have been found with a similar mutation. The Gly336Ser mutation creates a new restriction site for FspI. In order to explore whether this mutation could have arisen from a founder mutation within the French-Canadian PD population, we screened 102 French-Canadian patients with definite PD and 45 French-Canadian controls by PCR and restriction enzyme digestion. Oligonucleotide primers covering the NF-M G1747A mutation were designed according to the reported primer sequences. PCR products were digested with the restriction enzyme FspI and then electrophoresed on agarose gel. None of the patients nor controls had this mutation, indicating that this mutation is not common even in a PD population of similar ethnic background and suggests this change could represent a rare polymorphism. However, these results do not exclude the possibility that other mutations in this gene could be present.

**Association of *CCR5* and *CCL5* polymorphisms with Coal Workers' Pneumoconiosis.** R. NADIF<sup>1</sup>, A. JEDLICKA<sup>2</sup>, M. MINTZ<sup>2</sup>, F. KAUFFMANN<sup>1</sup>, S. KLEEBERGER<sup>3</sup>. 1) INSERM U472, VILLEJUIF, FRANCE; 2) Johns Hopkins University, MD, USA; 3) NIEHS, Research Triangle Park, NC, USA.

The role of C-C chemokines and their receptors in the pathogenesis of human lung inflammatory diseases is not clearly understood. The effect of functional polymorphisms in *CCR5* and its ligand *CCL5*, and their interactions with environmental exposure, were investigated in Coal Workers' Pneumoconiosis (CWP), a chronic inflammatory respiratory disease. In a longitudinal epidemiological study, 212 coal miners (aged 34-50 years in 1990) from the Lorraine Bassin (France) were genotyped for polymorphisms in *CCR5* (32 bp deletion) and *CCL5* (C-28G). Chest *x* rays scored according to International Labor Office classification of CWP, and subclinical computed tomography (CT) scores were performed in 1990 and 1994. Cumulative and current exposure to coal mine dust and smoking were recorded. *CCR5* 32 polymorphism was significantly associated with CT score in 1990 (1.27 1.83 vs. 2.12 2.27 p=0.01) and in 1994 (1.79 2.37 vs. 2.98 3.52 p=0.01), miners with wt/32 or 32/32 genotype having the highest score. No significant interaction with occupational exposure was found. *CCL5* -28 polymorphism was unrelated to CT score. Associations of *CCR5* 32 polymorphism with CT score in 1990 and in 1994 remained significant after adjustment for occupational exposure. The difference in adjusted CT scores according to *CCR5* genotype was 2.2 greater in miners with *CCL5* CG or GG genotype than in those with CC genotype of the ligand, but a formal test for interaction was not statistically significant. Significant interactions of *CCR5* 32 polymorphism with current smoking on CT score in 1990 and in 1994 were observed (p=0.03 and p=0.04), with CT scores significantly higher in smoker or ex-smoker miners with *CCR5* wt/32 or 32/32 genotype, whereas no association was found in non smokers. Results suggest the role of *CCR5* 32 polymorphism in the etiology of CWP with a potential interaction with smoking. Studies considering simultaneously *CCR5* with its ligand *CCL5* are of interest in inflammatory diseases.

**A pharmacogenetic study of response to a 5-lipoxygenase inhibitor in a Phase II clinical trial.** *S. Lewitzky<sup>1</sup>, G. Barnes<sup>2</sup>, P. Garg<sup>3</sup>, J. Metivier<sup>1</sup>, M. Chang<sup>3</sup>, B. Rosengren<sup>3</sup>, J. Meyer<sup>1</sup>.* 1) Dept of Molecular Epidemiology, Millennium Pharmaceuticals Inc., Cambridge, MA; 2) Dept of Molecular and Cellular Pharmacology, Millennium Pharmaceuticals Inc., Cambridge, MA; 3) Dept of Clinical Development, Millennium Pharmaceuticals Inc., Cambridge, MA.

LDP-977 is a candidate asthma drug that inhibits leukotriene production by antagonizing the enzyme 5-lipoxygenase. We hypothesized that clinical response to LDP-977 is associated with genetic polymorphisms. In a sample of 137 patients in a Phase II clinical trial, 136 polymorphisms in 39 candidate genes were genotyped and tested for association with response to LDP-977 in 99 Caucasian patients. Response was defined as achieving a 20% increase in forced expiratory volume in one second (FEV) from baseline to 2 hours after dosing; nonresponse was defined as failing to achieve a 15% increase. The 39 candidate genes include 14 genes in the leukotriene pathway (including 5-lipoxygenase), 7 drug metabolizing enzymes, and 18 genes in other known inflammation pathways. The 136 polymorphisms include 131 SNPs, 4 insertion/deletions, and 1 microsatellite. Each polymorphism was tested for evidence of pharmacogenetic association by standard single-point association tests, both allele-based and genotype-based. Each gene was also tested for evidence of pharmacogenetic association by a novel haplotype association test for unphased genotype data based on the principle that haplotype similarity should be greater within a response group than between response groups. Several tests yielded nominally significant p-values ( $p < .05$ ). A summary of the statistical results is presented.

**Asthma pharmacogenetics: Association of sequence variants in CRHR1 and improved lung function in asthmatics treated with inhaled corticosteroids.** *K.G. Tantisira<sup>1</sup>, S.L. Lake<sup>1</sup>, E.S. Silverman<sup>1</sup>, R. Lazarus<sup>1</sup>, E.K. Silverman<sup>1</sup>, S.B. Liggett<sup>2</sup>, S. Gabriel<sup>3</sup>, E. Lander<sup>3</sup>, J.M. Drazen<sup>1</sup>, S.T. Weiss<sup>1</sup>.* 1) Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; 2) Pulmonary Division, University of Cincinnati, Cincinnati, OH; 3) Whitehead Institute, Massachusetts Institute of Technology, Cambridge, MA.

The response to treatment of asthma shows high inter-individual variability. We hypothesized that a significant proportion of the variation in response to inhaled corticosteroids, the most commonly used asthma controller therapy, could be genetic. We genotyped 131 single nucleotide polymorphisms (SNPs) from 14 genes related to the biological action of inhaled steroids to evaluate the pharmacogenetic effect of treatment in an eight week clinical trial of 470 adults with moderate-to-severe asthma. Single SNP analyses revealed three genes with an association with the eight week change in lung function, as measured by the forced expiratory volume in one second (FEV1). We subsequently genotyped these three genes in DNA from 308 childhood asthmatics treated with inhaled corticosteroids. One gene, corticotropin releasing hormone receptor 1 (CRHR1, NM\_000756), demonstrated multiple single SNP associations within each of the populations. Three CRHR1 SNPs captured most of the haplotypic variation in both populations. One haplotype derived from these three SNPs was associated with over twice the lung function response in both populations ( $p = 0.02$  and  $0.04$  for the adults and children, respectively) compared to absence of this haplotype. Finally, we genotyped those three CRHR1 SNPs in a third clinical trial sample of 339 adult asthmatics and found a strong positive association with response to inhaled corticosteroids. Replication of the initial association between CRHR1 variation and response to inhaled corticosteroids in two additional independent samples of asthmatics may provide opportunities for improved asthma therapy planning.

**Nicotinic acetylcholine receptor gene polymorphisms, adolescent smoking, and attention deficit hyperactivity disorder (ADHD) subtypes.** *R.J. Neuman, E.A. Lobos, C.A. Joyner, H.-C. Ji, L. Sun, W. Reich, R.D. Todd.* Washington Univ Med Ctr, St Louis, MO.

Recent population based studies of ADHD have demonstrated the existence of heritable latent class subtypes of ADHD (Todd et al., 2001; Rasmussen et al., in press). In addition, associations between cigarette smoking and inattentive ADHD symptoms have frequently been reported in clinic-based studies. Using systematic mutation screening approaches, we found a significant association of an exon2/intron 2 junction polymorphism of the neural nicotinic acetylcholine receptor alpha-4 subunit gene, CHRNA4 with the latent class defined inattentive ADHD subtype (Todd et al, 2003).

We used logistic regression to test for an association between regular smoking with DSM and latent class ADHD subtypes while controlling for age, sex, ethnicity, zygosity, and comorbid traits (e.g., conduct disorder, alcohol abuse and marijuana use, major depression) in 800 male, female and opposite sex twin pairs recruited via Missouri birth-records. After correcting for comorbid conditions and other variables, there was a strong association of the latent class defined inattentive ADHD ( $p=0.002$ ) with regular smoking. Correcting for covariates the odds ratio for regular smoking in latent class inattentive ADHD was 4.7 (95% CI 1.8-12.4). However, no significant association of regular smoking was found with the DSM-IV defined primarily inattentive ( $p=0.10$ ) or combined subtypes ( $p=0.38$ ) ADHD. There was a weak association with the latent class defined combined subtype ADHD ( $p=0.04$ ). The average age of onset of smoking was not significantly different for this subtype of ADHD contrasted with non-ADHD controls (12.3 years for each). Although our current ADHD subtype sample size is not large enough to definitively implicate abnormalities in nicotinic genes for increased tobacco use in the latent class inattentive ADHD subtype, the current findings are compatible with the strong association between latent class inattentive ADHD subtype and smoking being related to polymorphisms in nicotinic acetylcholine receptor genes.

**Domains of speech-sound disorder and dyslexia are pleiotropically influenced by chromosome 3 region.** *C.M. Stein<sup>1</sup>, J.H. Schick<sup>1</sup>, H.G. Taylor<sup>2</sup>, L.D. Shriberg<sup>3</sup>, A. Kundtz-Kluge<sup>1</sup>, C. Millard<sup>1</sup>, K. Reading<sup>1</sup>, N. Minich<sup>2</sup>, A. Hansen<sup>2</sup>, L.A. Freebairn<sup>2</sup>, R.C. Elston<sup>1</sup>, B.A. Lewis<sup>2</sup>, S.K. Iyengar<sup>1</sup>.* 1) Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Pediatrics, Rainbow Babies & Childrens Hospital, Case Western Reserve Univ, Cleveland, OH; 3) Waisman Center on Mental Retardation and Human Development, Univ. of Wisconsin-Madison, Madison, WI.

Speech-sound disorders (SSD) are characterized by deficiencies in articulation, phonologic coding and memory, and language. It has recently been proposed that these early problems in spoken language predict development of dyslexia in later years. The correlation between speech-sound and dyslexia phenotypes suggests that these disorders may share some common genetic etiology. A previous study identified a candidate region on chromosome 3 that was linked to developmental dyslexia in a large Finnish family. We hypothesized that this region may be linked to domains shared by SSD and dyslexia. We utilized quantitative test scores that measure several processes underlying speech-sound production, including articulation, phonologic coding, and phonologic memory, to examine linkage to markers in the pericentrometric region of chromosome 3 in 77 families ascertained through a child with SSD. We also assessed linkage to traits measuring language and reading skills. Model-free linkage analysis was followed by identification of sibpairs that contributed most to the linkage effect, and construction of core shared haplotypes in these sibpairs. Our multipoint linkage analyses of measures associated with phonologic coding/decoding demonstrate the strongest linkage (D3S2465,  $P=5.6 \times 10^{-5}$ ; D3S3716,  $P=6.8 \times 10^{-4}$ ). The corresponding dyslexia phenotypes also demonstrated linkage (single word reading, D3S2465,  $P=.004$ ; nonsense word reading, D3S1595,  $P=.005$ ). The minimum shared haplotype in sibpairs with similar trait values spans 4.9 cM and is bounded by markers D3S3049 and D3S3045. Our results suggest that phonologic skills, which predispose to dyslexia, are influenced by a quantitative trait locus on chromosome 3, implying a pleiotropic effect on both disorders.

**Disentangling the autism phenotype; a sib-pair analysis.** *P. Szatmari*<sup>1</sup>, *C. Merette*<sup>2</sup>, *C. Emond*<sup>2</sup>, *L. Zwaigenbaum*<sup>3</sup>, *M. Maziade*<sup>2</sup>, *MA. Roy*<sup>2</sup>, *R. Palmour*<sup>4</sup>. 1) Dept Psychiatry, McMaster Univ, Hamilton, ON, Canada; 2) Dept of Psychiatry, Laval Univ, Quebec City, PQ, Canada; 3) Dept of Pediatrics, McMaster Univ, Hamilton, ON, Canada; 4) Dept of Psychiatry, McGill Univ, Montreal, PQ, Canada.

There are several dimensions that show familial aggregation among sib pairs with autism spectrum disorders. These include non-verbal IQ and communication skills. Since, these skills are highly correlated, it is not clear whether variation in IQ and communication arise from a common genetic background or from independent genetic mechanisms. We assembled 80 sib pairs with an autism spectrum disorder such as autism, Asperger syndrome and atypical autism based on standard diagnostic instruments and best-estimate. Within affected individuals there was a strong correlation ( $r=0.70$ ) between non-verbal IQ (as measured by the Leiter performance scale) and communication (as measured by the Vineland Communication Scale). In addition, there was strong familial correlation between sibs from the same family on both measures; intraclass correlations of 0.37 and 0.34, respectively. To see whether, the familial correlation on one measure was accounted for by the familial correlation on the other, we used multiple regression models. First, we calculated residual communication scores for each member of the sib pair based on their IQ score controlling for the age and gender of the affected individual. The process was repeated using IQ as the dependent variable and communication scores, along with the covariates, as the independent variables. These residual scores were then used to calculate intraclass correlations (ICC) between affected sibs. Strong familial correlations were still seen on the residual scores indicating that non-verbal IQ (ICC=0.39) and communication (ICC=0.36) may arise from independent genetic mechanisms in autism spectrum sib pairs. These data suggest that non-verbal IQ and communication should be tested as separate quantitative phenotypes in linkage analysis of autism sib-pairs, a strategy may improve power to find autism susceptibility genes.

**Autoimmunity related genes and their evolutionary fate as reflected in three inbred ethnic groups.** *I. Grossman*<sup>1</sup>, *C. Singer*<sup>2</sup>, *N. Avidan*<sup>2</sup>, *M. Chemla*<sup>2</sup>, *A. Miller*<sup>1</sup>, *D. Lancet*<sup>2</sup>, *J.S. Beckmann*<sup>2</sup>. 1) Division of Neuroimmunology and Multiple Sclerosis Center, Technion Israel Institute of Technology, Haifa; 2) Department of Molecular Genetics, Crown Human Genome Center, Weizmann Institute of Science, Rehovot, Israel.

To elucidate conservational forces shaping autoimmunity related genes, we examined the frequency of Single Nucleotide Polymorphisms (SNPs) in such genes in three healthy inbred Israeli ethnic groups. We considered genes reflecting, amongst others, immunological pathways common to autoimmune conditions, including cytokines, chemokines, apoptotic agents, proteases, etc. A total of 73 SNPs representing a diverse range of minor allele frequencies in each gene were selected from public databases, and typed using the Sequenom MassArray in Ashkenazi Jews, Sephardic Jews and Israeli-Palestinians. Allele frequencies of both SNPs and inferred haplotypes were compared by various statistical methods. The allele frequency of only one SNP was statistically significant in discerning the three groups from one another. However, comparison of inferred haplotypes showed significant distinction in 14 out of the 22 genes tested. These results suggest that a small set of SNPs with varied minor allele frequencies in each gene captures the greater part of the haplotypic diversity, even across recently diverged populations. The described strategy in SNPs selection may also be advantageous in association studies to seize differences between cases and controls. Though all the selected genes might be crucially involved in etiology of a vast variety of inflammatory and neuroimmunological diseases, allele frequencies in the above ethnic groups are generally unknown. The present study allows us to look for possible epistatic effects in autoimmunity related genes, according to population evolution. The pattern emerging from the results suggests that genes in processes more environmentally influenced, such as lipid metabolism and antiviral immunity, exhibit different haplotypic patterns in each ethnic group. In contrast, genes involved in more general pathways, such as proteases and apoptotic agents, show substantial similarity across all groups.

**Investigation of genotype frequencies of candidate genes for coronary atherosclerosis in dependence of the sexual hormone status in healthy female blood donors.** C. Glaeser<sup>1</sup>, S. Schulz<sup>1</sup>, U. Mueller-Werdan<sup>2</sup>, K. Werdan<sup>2</sup>, I. Hansmann<sup>1</sup>. 1) Inst Human Genetics, Univ Halle, Halle, Germany; 2) Dep Internal Med, Univ Halle, Halle, Germany.

Atherosclerosis is a very complex disease influenced by a variety of factors. Among this the sexual hormone status is established to be an important modulator of CAD. Further established genetic risk markers are involved in processes leading to the development of CAD like e.g. PM in the ACE-, ApoE-, CD-14- and E-selectin-gene as well as the Leiden-PM in the factor V-gene. We investigated the genotype distribution of these PM in a group of 174 healthy female blood donors without any coronary risk factors (> 3y in medical monitoring; mean: 39.7y). The group was divided into 1 group of 118 young women (<45y) whose hormone level is not already altered corresponding to their age and in a second group of 56 elderly women (>45y) who are adjusted hormonally in terms of age. For evaluation of the genotype distributions the DD- (ACE), the 3/4- (ApoE), the TT- (CD-14), the Arg/Arg+Arg/Ser- (E-selectin) as well as the AA+AG-genotype (factor V) were considered pathological in terms of coronary atherosclerosis in common consent. Investigating the genotype frequencies in dependence of the hormone status we could not detect any significant differences in the score of so called pathological genotypes between the hormone protected young and the elderly group (ACE: p=0.49; Apo-E: p=0.61; CD-14: p=0.54; E-selectin: p=0.68; factor V: p=0.38). Since it is well established that there is a first order cardioprotective effect of female sexual hormones like e.g. oestrogen it is conceivable that the number of pathological risk markers in healthy female persons who are no longer protected by their hormones should be obviously decreased because of the supposing higher cardiovascular susceptibility of persons carrying these genotypes. 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 PM could be markers for the progress and therefore for a practicable clinical therapy of the complex CAD.

**Resequencing analysis of the Mannose-binding lectin gene (MBL2) demonstrates substantial genetic diversity with functional implications.** *T. Bernig*<sup>1</sup>, *B. Staats*<sup>2</sup>, *M. Yeager*<sup>2</sup>, *J.G. Taylor*<sup>1</sup>, *C.B. Foster*<sup>3</sup>, *S. Chanock*<sup>1</sup>. 1) Pediatric Oncology Branch, National Cancer Institute-ATC, Gaithersburg, MD; 2) Core Genotyping Facility, National Cancer Institute-ATC, Gaithersburg, MD; 3) Johns Hopkins University, Department of Pediatrics, Division of Infectious Diseases, Baltimore, MD.

The human mannose-binding protein (MBL) is an important component of the innate immune system that protects organism against pathogens in the first line of host defence preceding the beginning of the adaptive immune response by T and B-lymphocytes. There are seven described secretor haplotypes of the MBL2 gene based on 3 strongly linked promoter SNPs and a set of non-synonymous SNPs (known as B, C and D) in exon 1, which influence both serum levels and functional activity. Overall, select haplotypes, which correlate adequately with levels have been associated with increased susceptibility to a spectrum of infections and complications of immunodeficiency states; interestingly, these variants are also protective against other infections (i.e. tuberculosis). Complete resequencing analysis of MBL2 revealed that it is highly polymorphic with 87 variants across the gene (about 10.0 kb) in 4 separate populations: African American, Caucasian, Hispanic and Pacific Rim. Analysis of linkage disequilibrium appears to be complete across the entire gene but shows distinct differences between Caucasians and African Americans, with greater complexity in both structure and distribution of haplotypes in the latter. Moreover, there is evidence for gene conversion in the 3 region of the gene that disrupts LD in the latter. This suggests a functional implication for the 3 region and could have substantial implications for investigation of the importance of genetic variation within MBL2 in disease. These findings imply that future association studies with MBL2 require a locus wide approach with a much denser map of polymorphic sites and that the exclusive use of the classical secretor haplotypes is insufficient to detect the diversity in MBL2. Lastly, we have determined that the B, C and D structural alleles (which are central to the 7 secretor haplotypes) lie on restricted haplotypes, suggesting that they have arisen recently.

**IL10 polymorphisms and risk of cardiovascular disease in the CHOICE cohort.** *Y. Berthier-Schaad*<sup>1, 2</sup>, *L. Plantinga*<sup>1</sup>, *Y. Liu*<sup>1</sup>, *N. Fink*<sup>1</sup>, *M.J. Klag*<sup>1</sup>, *J. Coresh*<sup>1</sup>, *M.W. Smith*<sup>3</sup>. 1) Johns Hopkins Medical Institutions, Baltimore, MD; 2) Laboratory of Genomic Diversity, NCI, Frederick, MD; 3) Basic Research Program, SAIC, NCI, Frederick, MD.

Accumulating evidence suggests that inflammation plays an important role in the development of cardiovascular disease (CVD). IL10 is an anti-inflammatory cytokine and polymorphisms in the IL10 promoter region have been associated with lower IL10 and inflammatory markers levels and decreased risk of CVD in some but not all studies. We tested the relationship between IL10 genotypes, inflammatory markers levels and CVD incidence in a cohort of 1041 dialysis patients where subclinical inflammation is common and strongly predictive of CVD. Using a 5'exonuclease assay, genotypes were obtained for six loci: -3575 T/A, -1082 A/G, and -592 C/A in the promoter region, 4467 G/A in intron 4, 5016 T/C and 5351 A/G in the 3' UTR region. Haplotypes were estimated using resequencing data and PHASE analysis of the genotypes. Adjusted differences of inflammatory markers and relative hazards of CVD and mortality associated with IL10 variants were calculated overall as well as stratified by race and smoking history. There was no association between any polymorphism and relative hazard of CVD over the entire population. However, in race-stratified analyses, alleles -1082G and 5016C were associated with higher levels of IL6 and CRP in patients of European descent (ED) ( $P < 0.05$ ), while alleles -3575A and 4467A were associated with lower levels of IL6, CRP and fibrinogen in patients of African descent (AD) ( $P < 0.05$ ). One haplotype was associated with lower relative hazard of CVD [RH 0.31 (0.12-0.83)] in AD patients. In analyses stratified by smoking history, there was no association between any haplotype and relative hazard of CVD. We find no strong and constant association between the six IL 10 variants and the levels of inflammatory markers and CVD incidence. The slightly protective effect of one haplotype in AD patients is consistent with previous literature. Funded in part by RO1 L62985, AHA EI01-40197ND & DHHS#N01-CO-12400.

**T102C 5-Hydroxytryptamine (5-HT<sub>2A</sub>) receptor polymorphism in late-onset cardiovascular diseases and its risk factors.** *I.B.M. DaCruz<sup>1</sup>, C.H.A. Schwanke<sup>2</sup>, M. Taufer<sup>1</sup>, G. Oliveira<sup>1</sup>, L. Bittencourt<sup>2</sup>, G.A.L. Flores<sup>1</sup>, A.A.M. Cruz<sup>1</sup>, J. Siviero<sup>1</sup>, I.E.C. Jung<sup>1</sup>, E.H. Moriguchi<sup>1</sup>, GENESIS RESEARCH PROGRAM.* 1) IGG, PUCRS, Porto Alegre, RS, Brazil; 2) Faculdade de Biociencias, PUCRS, Porto Alegre, RS, Brazil.

Serotonin (5-HT) is involved in cardiovascular pathophysiology. In addition to platelet aggregation and vascular contraction, 5-HT induces hyperplasia of artery smooth muscle cells. Previous studies suggested that the T102C (5-HT<sub>2A</sub>) polymorphism is related to cardiovascular and psychiatric disorders. However, the data are still controversial. This case-control study was designed to analyse possible associations between DNA polymorphisms in the serotonin (5-HT<sub>2A</sub>) receptor with late-onset (>60 years) cardiovascular diseases and risk factors. 725 living community subjects were recruited from Genesis Research Program that investigate gene-environment interaction on aging and related diseases in the Brazilian population. The T102C polymorphism was detected by PCR-RFLP using HpaII restriction enzyme. The average age was 68.056.35 years. The genotype frequency was TT= 22.9 (167), CC=21.8 (158) and CT=55.2 (400). The allelic frequency was T=0.506 and C=0.494. The frequencies were in Hardy-Weinberg equilibrium. No cardiovascular disease association (myocardial infarction, angina pectoris and stroke) with the T allele was found. However we found CC independent risk association with DM [OR=2.16 (1.10-4.28)]; obesity [OR=1.69 (1.00-3.11)] and smoking habit [OR=1.63 (1.10-2.51)]. The associations could be better explained by the neurological role other than by the vascular role of the 5-HT<sub>2A</sub>. Additionally, it is important to consider that serotonin metabolism studies have been reporting association with several clinical conditions as early-onset no-fatal myocardial infarction, hypertension, eat disorders and compulsive behaviour. Also, the serotonin re-uptake drugs are extensively used in clinical setting. Therefore, complementary studies could help to elucidate the complex associations reported in the literature and here.

**Risk of female breast cancer and the *BRCA2* N372H polymorphism: a population-based case family study.** *M.A. Jenkins<sup>1</sup>, R.L. Milne<sup>1</sup>, M.C. Southey<sup>1</sup>, A.B. Spurdle<sup>2</sup>, G. Chenevix-Trench<sup>2</sup>, J.L. Hopper<sup>1</sup>, Australian Breast Cancer Family Study.* 1) University of Melbourne, Australia; 2) Queensland Institute of Medical Research, Brisbane, Australia.

Two large case-control studies have shown that the *BRCA2* 372 *HH* genotype was associated with a 30% to 50% increased risk of female breast cancer. We have used a population-based case family study, the Australian Breast Cancer Family Study, to estimate the age-specific risks of breast cancer in carriers of this genotype. Genotyping was conducted on 1353 women diagnosed with breast cancer before age 60 years (half under 40 years) who were incident cases sampled from cancer registries, and 1827 of their adult first- and second-degree relatives. Families with an identified carrier of a *BRCA1* or *BRCA2* mutation were excluded. Personal and family history of breast cancer was obtained from all participants and attempts were made to validate all cancer reports. The hazard ratio (ratio of age-specific incidence of breast cancer in women with the *HH* genotype (carriers) to that in women without the genotype (non-carriers)) and the age-specific cumulative risk (penetrance) were estimated by a modified segregation analysis. Familial aggregation was modelled by adjusting for the breast cancer status of each individual's mother. Models were fitted under maximum likelihood theory using the statistical package MENDEL. The allele frequency was fixed at 0.27 based on 775 controls. Under a recessive model (superior fit to dominant model), women with the *HH* genotype were 1.3 (95% CI 1.1 - 1.6) times more likely to be diagnosed with breast cancer, independently of a 2.5 (1.8 - 3.4) fold risk for having an affected mother. The cumulative risks of breast cancer to age 70 were: 5.7% (non-carriers with unaffected mother); 7.3% (carriers with unaffected mother); 13.3% (non-carriers with affected mother); and 16.9% (carriers with affected mother). These results, using family data that are not subject to population stratification, confirm that the allele is associated with a small, recessively inherited increased risk of breast cancer, or is in linkage disequilibrium with a disease predisposing variant.

**Association of angiotensin I-converting enzyme polymorphisms with obesity in Nigerians, Jamaicans, and African Americans.** *Y. Jiang<sup>1</sup>, H. Kramer<sup>1</sup>, X. Zhu<sup>1</sup>, A. Luke<sup>1</sup>, N. Bouzekri<sup>2</sup>, C. Mckenzie<sup>3</sup>, T. Forrester<sup>3</sup>, A. Adeyemo<sup>4</sup>, M. Farrall<sup>5</sup>, S. Anderson<sup>2</sup>, R. Cooper<sup>1</sup>, R. Ward<sup>2</sup>.* 1) Department of Preventive Medicine and Epidemiology, Stritch School of Medicine, Loyola University, Maywood, IL, USA; 2) Department of Biological Anthropology, University of Oxford, Oxford, UK; 3) Tropical Metabolism Research Institute, University of the West Indies, Mona, Jamaica; 4) Department of Pediatrics/Institute for Child Health, College of Medicine, University of Ibadan, Nigeria; 5) Department of Cardiovascular Medicine, University of Oxford, The Wellcome Trust Center for Human Genetics, Oxford, UK.

We report an association analysis of obesity with the angiotensin I-converting enzyme (ACE) polymorphisms in Nigerians, Jamaicans, and African Americans. Thirteen single nucleotide polymorphisms (SNPs) expanding 25 kb of the ACE gene were genotyped in each of the three population samples. Fifteen major haplotypes were grouped into 4 clads, I, II, III, and IV, from the most frequent to the least frequent, by Templeton algorithm (Templeton et al. 1987, 1988). Haplotype analysis of multiple SNPs was conducted by TRANSMIT (Clayton 1999) which contrasts the observed number versus the expected number of a haplotype (under H<sub>0</sub>) transmitted from parents to his or her obese children. Different sets of multiple SNPs were found in each of the three population samples. Each population sample has two significant haplotypes ( $p < 0.06$ ), one was over transmitted and the other was under transmitted. The over transmitted haplotype belongs to clad I and/or IV. The under transmitted haplotype belongs to clad II and/or clad III except the one in the African Americans with very low frequency. The common SNP in the three sets is ACE8 which has the strongest association with ACE levels among the 13 SNPs genotyped in all three samples. In conclusion, we found some evidence of association of the ACE polymorphisms with obesity in three black population samples through a cladistic analysis using multiple ACE polymorphisms.

**Genetic variation in TNF (G-308A) is associated with measures of body composition in older women.** *S.P. Moffett<sup>1</sup>, J.M. Zmuda<sup>1</sup>, J.A. Cauley<sup>1</sup>, K.L. Stone<sup>2</sup>, M.C. Nevitt<sup>2</sup>, K.E. Ensrud<sup>3</sup>, M.C. Hochberg<sup>4</sup>, T.A. Hillier<sup>5</sup>, G. Joslyn<sup>6</sup>, P. Morin<sup>6</sup>, S.R. Cummings<sup>2</sup>, for the SOF Research Group.* 1) Univ Pittsburgh, Pittsburgh, PA; 2) Univ California, San Francisco, CA; 3) Veterans Affairs Medical Center, Minneapolis, MN; 4) Univ Maryland, Baltimore, MD; 5) Kaiser Permanente, Portland, OR; 6) Axys Pharmaceuticals, CA.

Tumor necrosis factor (TNF) is a proinflammatory cytokine that regulates adipose, muscle and bone metabolism and thus has been targeted as a potential candidate gene for alterations in body composition. The G-308A polymorphism in the TNF promoter has been associated with alterations in mRNA and plasma levels where the A allele corresponds to increased TNF. In the present study, we evaluated the relationship between the TNF G-308A polymorphism and measures of body composition in 3376 women aged 65 years and older participating in the Study of Osteoporotic Fractures. The frequency of the -308A allele was 0.167. Using analysis of covariance, we found that women with the AA genotype were significantly taller and heavier than the GG homozygotes (mean height in cm (standard error): GG=158.5 (0.1), GA=159.1 (0.2), AA=159.7 (0.6),  $p=0.003$ ; weight in kg: GG=66.4 (0.3), GA=67.5 (0.4), AA=68.2 (1.3),  $p=0.034$ ). These relationships remained significant after adjusting for study center, age, current estrogen use, health status, exercise, steroid use and smoking. Fat mass (FM) and fat free mass (FFM), measured using bioelectrical impedance, were also higher in the AA homozygotes; however, FM did not reach statistical significance when the three genotypes were considered separately (FM in kg: GG=26.6 (0.2), GA=27.4 (0.3), AA=27.6 (0.9),  $p=0.061$ ; FFM in kg: GG=39.8 (0.1), GA=40.2 (0.1), AA=40.5 (0.5),  $p=0.032$ ). In addition, AA homozygotes had higher spine and hip BMD (measured with dual energy x-ray absorptiometry) compared to the GG women (spine in  $g/cm^2$ : GG=0.852 (0.004), GA=0.876 (0.006), AA=0.874 (0.018),  $p=0.002$ ; hip in  $gm/cm^2$ : GG=0.756 (0.003), GA=0.760 (0.004), AA=0.766 (0.014),  $p=0.586$ ), but findings reached significance at the spine only. Our results support a link between allelic variation at the TNF locus and body composition regulation.

**Nucleotide diversity in the *DC-SIGN* gene.** *J.J. Martinson*<sup>1</sup>, *C. Eddy*<sup>2</sup>, *J. Chambers*<sup>1</sup>, *C. Bannerjee*<sup>1</sup>. 1) Inf. Diseases & Microbiology, University of Pittsburgh, Pittsburgh, PA; 2) Institute of Genetics, University of Nottingham, Nottingham UK.

Dendritic Cells (DCs) are highly specialized antigen-processing cells that can activate both naive and memory T-lymphocytes, and play a pivotal role in the initial presentation of many of pathogens, including HIV, ebola virus and mycobacteria. Both processes require the involvement of the DC-specific ICAM-grabbing nonintegrin protein DC-SIGN. This protein has recently been shown to be of critical importance in the early stages of cellular infection with HIV, as virus particles bound to DC via DC-SIGN are maintained in a viable state for long periods of time, and can infect target cells in trans.

Much is now known about the genomic organization of the *DC-SIGN* gene, its closely-related homolog *DC-SIGNR*, and other similar genes in primates. The gene is known to be alternately spliced, producing proteins with differences in lineage tropism, and possibly in cellular localization. Despite this, little is known sequence variation within the gene and the effect such nucleotide diversity may have on the ability of the DC-SIGN protein to bind pathogens and interact with T-lymphocytes. We present data here on an initial survey of sequence variation in the *DC-SIGN* gene in a random sample of Caucasian and Asian individuals.

The *DC-SIGN* gene consists of 1202bp in seven exons located in a 4.4kb region. We obtained full sequence data on each exon, and a 1840bp of intron sequence, from a total of 90 individuals. We found a total of 17 SNPs, evenly distributed between coding and noncoding regions. Four coding SNPs were found, in exons 4, 6 and 7, and all of these altered the amino acid sequence of DC-SIGN. Minor allele frequencies ranged from singleton observations to 14%, with the commonest SNPs located in the introns. The estimated nucleotide diversity was higher than observed for many genes, being  $10^{-3}$  for the exons and  $1.45 \times 10^{-3}$  in the introns. Thus there is a great deal of nucleotide diversity within *DC-SIGN*, including many coding variants whose biological significance remains to be elucidated.

**Allele frequencies of the short and long tetra-nucleotide repeat [TTTA] n in the CYP-19 gene in African-American and Caucasian women.** *C. Lara-Castro, U. Desai, S.M. Sell.* Nutrition Sciences, Univ Alabama, Birmingham, AL.

A tetranucleotide repeat polymorphism in the fourth intron of the CYP19 gene is associated with different conditions in women. The short allele is associated with increased risk of osteoporosis and breast cancer. The longer allele (187 bp) is associated with decrease risk of obesity in women. Because the burden of these diseases is different among women of different ethnic groups, it is important to determine allele frequencies of polymorphisms in relevant candidate genes, such as the CYP19 gene, across women of different ethnic backgrounds. To determine differences in allele frequencies of the short (167bp) and long allele (187bp) of the CYP19 polymorphism in women of Caucasian and African-American backgrounds, DNA from a bi-ethnic sample (n=242) was genotyped. We are reporting preliminary data on 36 normal controls (12 Caucasian and 24 African-American). MapPair primers, specific for the CYP19 tetra nucleotide repeat were obtained and PCR amplification was conducted following their specifications. The larger allele (187bp) was more common than the shorter allele (167bp) in this group of women, 31% vs. 16%. Table 1 shows allele frequencies by ethnic group. These results suggest that frequencies of the short and long alleles in the aromatase gene varied between ethnic groups. Further research is necessary to determine the role of these differences as they relate to disease phenotypes in women. Table 1

Allele size (bp)	167 n (%)	187 n (%)
African-American (N=24)	10 (21)	9 (19)
Caucasian (N=12)	2 (8)	13 (54)
Total (N=36)	12 (17)	22 (31)

N=number of subjects, n=number of alleles.

**Human chromogranin A (CHGA): discovery of both frequent and rare functional polymorphisms at a locus governing catecholamine storage and release.** *G. Wen*<sup>1</sup>, *S.K. Mahata*<sup>1,2</sup>, *P. Cadman*<sup>1,2</sup>, *M. Mahata*<sup>1,2</sup>, *S. Ghosh*<sup>1,2</sup>, *N.R. Mahapatra*<sup>1,2</sup>, *F. Rao*<sup>1,2</sup>, *M. Stridsberg*<sup>3</sup>, *D.W. Smith*<sup>4</sup>, *P. Mahboubi*<sup>1,2</sup>, *D.T. OConnor*<sup>1,2</sup>, *B.A. Hamilton*<sup>1</sup>. 1) Department of Medicine,UCSD,La Jolla,CA; 2) VA San Diego Healthcare System, San Diego,CA; 3) Department of Medical Sciences, University Hospital, Uppsala, Sweden; 4) Division of Biology,UCSD,La Jolla,CA.

Chromogranin A (CHGA) is a central regulator of catecholamine vesicle storage and release. CHGA is also a candidate gene for autonomic dysfunction syndromes, including intermediate phenotypes that contribute to human hypertension. In order to identify genetic variants in CHGA that might alter its function, we resequenced all 8 exons and adjacent intronic regions, ~1.2 kbp of 5 promoter, and two intronic conserved noncoding regions from 180 ethnically diverse human subjects. We identified 53 single nucleotide polymorphisms (SNPs) and 2 single base insertion/deletions in this ~5.7 kb footprint. Of these, 20 SNPs were common in our samples. Surprisingly, 8 of the common SNPs occur within less than 1.2 kb in the proximal promoter. The estimated nucleotide diversity ( $\pi$ ) in the promoter region (0.0021) is ~3 fold higher than in other regions of CHGA. Tajimas T and H tests showed significant departure from neutrality in the promoter region, but not in other regions. Among these polymorphisms, we inferred 6 common haplotypes in promoter region. Promoter strength assessed in vitro is markedly reduced in two closely related haplotypes compared with all other haplotypes. Phylogenetic analysis shows that the high and low-expressing haplotype groups also mark the deepest division of the human lineage for this gene. Two amino acid substitutions, Gly364Ser and Pro370Leu, are rare variants in functionally important catestatin peptide. Interestingly, the Gly364Ser substitution results in a 4.7-fold loss of potency, while the Pro370Leu substitution produces a 2.3-fold gain of potency for inhibition of nicotine-evoked catecholamine secretion. Our data demonstrate that both common and rare variants contribute to functional variations in CHGA. Genetic and functional analysis suggests that the level of CHGA and catestatin is under positive selection.

**Angiotensin-Converting Enzyme Gene Polymorphism, Carotid Artery Wall Thickness and Cardiovascular Mortality; Findings from the Rotterdam Study.** *F.A. Sayed-Tabatabaei<sup>1</sup>, A. Arias-Vásques<sup>1</sup>, Y. Aulchenko<sup>1</sup>, A. Hofman<sup>2</sup>, J.C.M. Witteman<sup>2</sup>, C.M. van Duijn<sup>1</sup>.* 1) Genetic Epidemiology Unit, Department of Epidemiology & Biostatistics, Erasmus MC, Rotterdam, The Netherlands; 2) Department of Epidemiology & Biostatistics, Erasmus MC, Rotterdam, The Netherlands.

*Introduction:* Although angiotensin-converting enzyme (ACE) plays a major role in blood pressure regulation, the role of the gene in the risk of atherosclerosis and vascular mortality is unclear. We performed an association study on the insertion/deletion (I/D) polymorphism of the ACE gene and carotid intima media thickness (IMT) and risk of cardiovascular and total mortality in 6869 subjects from the Rotterdam Study. The follow-up data for overall mortality was assessed until the year 2003 and for cardiovascular mortality until the year 2000.

*Results:* There was a small increase in IMT in carriers of the D allele when analysed in the total 5394 subjects for whom IMT measurements were available. The association was limited to smokers. In current smokers there was a significant trend ( $p = 0.04$ ), with the highest IMT in the DD genotype (mean difference DD vs. II =  $0.26 \text{ mm} \times 10^{-1}$ ,  $p = 0.03$ ). The observed difference remained after adjustment for blood pressure.

During the follow-up, 2304 all-causes deaths and 575 cardiovascular deaths were recorded. The hazard ratio (HR) of total mortality in DD carriers compared to II carriers was increased only in smokers who had the event before age 75 (HR=1.52, 95% confidence interval (CI) 1.03-2.25,  $p=0.03$ ). An increased risk for the DD carriers compared to II carriers was also seen for cardiovascular mortality (HR=3.22, 95% CI 1.06-9.77,  $p=0.04$ ).

*Conclusion:* We found a positive association of the D allele of the I/D polymorphism with carotid IMT and risk of cardiovascular mortality among smokers. The findings suggest that the gene may be an important determinant of cardiovascular disease in smokers.

**The deletion/insertion polymorphism of the angiotensin converting enzyme gene and primary palmar**

**hyperhidrosis.** Z. Yamagata<sup>1</sup>, T. Kakinuma<sup>2</sup>, H. Uchino<sup>3</sup>. 1) Dept Health Sci, University of Yamanashi, Yamanashi, Japan; 2) Department of Anesthesiology, Tokyo Medical University, Tokyo, Japan; 3) Department of Anesthesiology Hachiohji Medical Center Tokyo Medical University, Tokyo, Japan.

Palmar hyperhidrosis (HH) is a distressing condition that can impair quality of life causing social, psychological, educational and occupational problems. It has been reported that the hyperactivity in skin sympathetic nerve activity of HH patients that involves contains sudomotor as well as vasoconstrictor nerve activity. Recently, it has been reported that chronic pain due to herpes zoster is associated with the polymorphism of the angiotensin converting enzyme gene and this gene could be a new landmark of the sympathetic function. In this study, we have explored the association between the ACE gene polymorphism and primary HH.

**Materials and Methods:** 40 consecutive patients (9 men, 31 women) with primary HH were included in the study. Serum ACE, angiotensin I, II, were measured in both groups. Blood samples were collected from both 40 patients and 40 healthy subjects. The insertion/deletion DNA polymorphism detected by human ACE.

**Result:** The mean age of HH subjects were 25.1 in II, 23.3 in ID, and 22.3 in DD. On the other hand, The mean age of controls healthy subjects were 26.1 in II, 26.1 in ID, and 25.1 in DD. The distribution of Genotypes in healthy subjects (II=0.20, ID=0.55, DD=0.25) and HH subjects (II=0.05, ID=0.425, DD=0.525) were in agreement with the Hardy-Weinberg proportion. Allele frequency of controls healthy subjects was 0.48 of I allele and 0.52 of D allele. In HH subjects, allele frequency was 0.26 of I allele and 0.74 of D allele. The D allele was significantly more frequent in HH subjects. The association was stronger in both homozygous and heterozygous patients (odds ratio DD/II was 8.4(95% CI, 1.49-47.04) and ID/II was 3.1(95% CI, 0.57-16.48), respectively. The ACE activity in DD genotype showed significant differences compared to the II and ID genotype of HH subjects.

**Genetic susceptibility and gastric cancer risk in a Colombian population.** *M.M. Torres, C.P. Acosta, D.M. Sicard, H. Groot de Restrepo, Laboratorio de Genética Humana. Departamento de Ciencias Biológicas, Universidad de los Andes, Bogota, Colombia.*

The main cause of cancer mortality in Colombia is due to gastric cancer (GC). The risk to develop GC has been associated with environmental factors and *Helicobacter pylori* (Hp) infection. The tumor necrosis factor (TNF- ) is a potent proinflammatory cytokine, and its levels are increased in patients infected with Hp. A G/A transition in the position -308 of the promoter of the TNF- has an increased expression of the gene, and this has been associated with the susceptibility of GC. Glutathione S-transferase (GST) enzymes are involved in the detoxification of many environmental carcinogens. The homozygous deletions of GSTT1 and GSTM1 have been associated with some types of cancer. In the present study, the association of genetic polymorphisms of TNF- at position -308 of the promoter and the deletion polymorphisms of GSTT1 and GSTM1 with GC were investigated in a south western region of Colombia. The interaction of these polymorphisms and other risk factors such as Hp infection, alcohol consumption, smoking and some dietary habits were explored. The information was collected in a questionnaire, and the blood samples were obtained from all the subjects: 50 patients with GC and 96 controls. The logistic regression model was used and the independent effect of each of the polymorphisms on the risk of GC and their potential interaction with other factors was assessed. The frequency of the G/A transition in the TNF- promoter was 18% in the GC population infected with Hp, and 7% in the control group. This transition was not significantly associated with Hp infection and GC. The frequencies of the deletion polymorphisms for both populations (patients and controls) respectively were as follows: GSTM1 65.2%; and 37.5%; GSTT1 17.4%; and 14.6%;. Our results suggest that the GSTM1 deletion polymorphism may be associated with an increased risk of gastric cancer (OR of 5.5; 95%;CI, 1.7-17.2). Also, our data showed that other risk factors such as Hp infection and alcohol intake are associated with this type of cancer (OR of 5.57; 95%;CI, 1.7-17.7; and 3.27; 95%;CI, 1.1-9.4) respectively.

**Association of the D2 dopamine receptor gene with opium-addiction in Iran.** *M. Shahmoradgoli N.<sup>1, 2</sup>, M. Ohadi<sup>1</sup>, F. Valaie<sup>1</sup>, Y. Riazalhosseini<sup>1</sup>, M. Mohammadbeigi<sup>1</sup>, H. Najmabadi<sup>1</sup>.* 1) Genetics Research Center, The Social Welfare and Rehabilitation Sciences University, Tehran, Iran; 2) Young Researchers Club, Azad University, Tehran, Iran.

Dysfunction of central Dopaminergic neurotransmission has been suggested to play an important role in the etiology of certain neuropsychiatric disorders such as drug abuse. It has been shown that the Dopamine D2 Receptor (DRD2) gene dysfunction is associated with multi-drug addiction. Addiction to opium is the most common form of drug abuse in Iran. We studied the allelic association between DRD2 Taq I A polymorphism in 100 opium-dependent patients and 80 unrelated controls. A 310 bp region surrounding Taq I site at the DRD2 locus was amplified by polymerase chain reaction (PCR) and the PCR product was incubated with Taq I restriction enzyme. The A1 allele remained intact while the A2 allele was cut. Significant association was observed between A1 allele and addiction in the patients group ( $P=0.0001$ ). Moreover, the frequency of A1A1 genotype was significantly higher in opium users than controls ( $P=0.0001$ ). Our result further indicates that DRD2 may be involved in the pathophysiology of opium addiction.

Program Nr: 1308 from 2003 ASHG Annual Meeting

**Characterization of human variation panels from African American, Caucasian, Han Chinese and Mexican American populations.** *L. Toji, P. Bender, C. Beiswanger, D. Coppock, J. Beck.* Coriell Cell Repositories, Coriell Inst Med Res, Camden, NJ.

Panels of 100 DNA samples isolated from cell cultures in the Human Genetic Cell Repository at the Coriell Cell Repositories from apparently healthy individuals of African American and Caucasian origins have been available for investigators studying human variation since January, 2000. Because of interest in larger sample sets, a second, non-overlapping set of 100 Caucasian samples has been identified from within the NIGMS collection and made available to investigators in the past year. To extend these panels to other major populations found in the US, blood samples have been collected from Chinese Americans and Mexican Americans under the "Policy for the Responsible Collection, Storage, and Research Use of Samples from Identified Populations for the NIGMS Human Genetic Cell Repository." Epstein-Barr virus-transformed lymphoblastoid cultures have been established and frozen, and DNA has been isolated from these anonymous samples. The Han People of Los Angeles Panel of 100 is a selection of 50 male and 50 female samples. Each sample is from an individual of Han ethnicity unrelated to all others in the Panel and has all four grandparents born in Taiwan, China, or Hong Kong. The Mexican-American Community of Los Angeles Panel of 100 is a selection of 50 male and 50 female samples. Each sample is from an individual unrelated to all others in the Panel and has either three or four grandparents born in Mexico. As part of the routine quality control procedure of the CCR, a set of six highly polymorphic microsatellite markers was determined for each of the 500 DNA samples in these panels. The cumulative profiles for each marker for each panel have been compared; the similarities and differences between panels will be discussed along with additional data on occurrence of the COII/tRNA(Lys) intergenic 9-bp mitochondrial DNA deletion in these populations. Information about these resources may be found at <http://ccr.coriell.org/nigms>.

**Val16Ala Polymorphism of the gene of the manganese(SOD2)-dependent enzyme Superoxide Dismutase, could contribute to increase the predisposition to age-associated diseases?** *M. Tauffer<sup>1</sup>, I.B.M. DaCruz<sup>1</sup>, A.L. Frasson<sup>3</sup>, C.H.A. Schwanke<sup>2</sup>, G. Oliveira<sup>1</sup>, A.R. Santos<sup>1</sup>, P. Jobim<sup>1</sup>, M.E.P. Canto<sup>1</sup>, D. Mussel<sup>1</sup>, J. Maia-Filho<sup>1</sup>, J.E. Piccoli<sup>1</sup>, L. Vieira<sup>1</sup>, I.E.C. Jung<sup>1</sup>, E.H. Moriguchi<sup>1</sup>.* 1) IGG, PUCRS, Porto Alegre, RS, Brazil; 2) Faculdade de Biociencias, PUCRS, RS, Brazil; 3) Faculdade de Medicina, PUCRS, Porto Alegre, RS, Brazil.

Several factors associated to aging stands out the crucial role that oxidative stress plays on macromolecules. This fact associated to differential responses of the anti-oxidant defense system, due to the presence of genetic polymorphisms, as is the case of the Val16Ala SOD2 polymorphism, could contribute to increase the predisposition to age-associated diseases. The association between the SOD2 Val16Ala polymorphism and chronic-degenerative diseases (cardiovascular-CVD and breast and prostate cancer-NEOPL) were investigated in a case-control study, with elderly participants of the Genesis Research Program (n=1158). To proceed, the following groups were selected: 1) healthy elderly individuals without diseases (HE) control group; and 2) elderly with cardiovascular diseases (ECVD) and breast and prostate cancer (NE) case group. Subjects with plurimorbidities were excluded. Besides, a control group of healthy adults was selected (HA). The SOD2 polymorphism was detected by PCR-RFLP using MspI enzyme restriction. The genotypic frequencies were AA=13%, VV=23.8% and AV=63.2%, and they were at Hardy-Weinberg equilibrium. In the control groups the frequency of AA individuals was significantly lower (HA=7.0% and HE=9.8%, NE=15.0%, ECVD=19.1%) (p=0.03). The AA genotype associated to the affected group (case), with an odds ratio of the individuals with the AA genotype presenting CVD and NEOPL of the 2.50 (1.24-5.05). The results suggest SOD2 polymorphism association as risk for these diseases. The present study also allowed the elaboration of mathematical models of possible association between the gene polymorphism, early and late diseases, and aging.

**The "Twins of mistaken zygosity" (TOMZ) study design: Evidence for a genetic contribution to dietary choices.**

*E. Gunderson*<sup>1</sup>, *A. Tsai*<sup>1</sup>, *M. Austin*<sup>2</sup>, *E. Mayer-Davis*<sup>3</sup>, *N. Risch*<sup>1,4</sup>. 1) Division of Research, Kaiser Permanente, Oakland, CA; 2) U Washington; 3) U South Carolina; 4) Stanford U.

Twin designs, comparing correlations in MZ versus DZ twins, have an extensive history. A major confounder in such studies is that MZ twins may share postnatal environmental influences more than DZ twins. To avoid such confounding, twins separated at or soon after birth have sometimes been studied, but their scarcity often makes this approach impractical. Another method has been to measure the degree of contact twins have maintained over time, and adjust observed correlations for degree of contact.

Here we propose another complementary approach to remove the effect of confounding in twin studies. Presumably, postnatal environmental sharing between twins is a function of their perceived zygosity. DNA zygosity testing of twin pairs has shown that about 10% of twins are mistaken in their self-perceived zygosity. This discrepancy between biological and self-perceived zygosity provides an opportunity to separate environmental from genetic sources of twin similarity.

We demonstrate the power of this method by an analysis of preferences for 15 food types obtained from 351 female twin pairs of the Kaiser Permanente female twin registry. In this group, 176 pairs were MZ by self-report and DNA testing (MZC); 136 pairs were DZ by self-report and DNA testing (DZC); 30 pairs were MZ by DNA but not by self-report (MZW); 9 pairs were DZ by DNA but not by self-report (DZW). The average intraclass correlations across 15 food types were MZC: .361.123; MZW: .334.166; DZC: .204.071; DZW: .258.296. These results show that correlations are largely determined by biological rather than self-perceived zygosity. Comparing MZC with DZW and MZW with DZC groups gives average heritability estimates of 21-26%; comparing MZC with MZW and DZW with DZC groups gives a component due to greater environmental sharing of MZ than DZ twins of 3-5%.

Studying twins of mistaken zygosity provides a powerful alternative approach to dealing with confounding within the classic twin design.

**Analysis of CFTR gene mutation frequency in a random African population.** *C. Bombieri*<sup>1</sup>, *B.M. Ciminelli*<sup>2</sup>, *F. Belpinati*<sup>1</sup>, *C. Ciccacci*<sup>2</sup>, *D. Salvi*<sup>2</sup>, *P.F. Pignatti*<sup>1</sup>, *G. Modiano*<sup>2</sup>. 1) Section Biol & Genetics, DMIBG, Univ Verona, Italy; 2) Dpt Biol E.Calef, Univ Roma-Tor Vergata, Italy.

In a previous collaborative research project we analysed Cystic Fibrosis Transmembrane Regulator (CFTR) gene variation among random European healthy subjects (Hum Genet 106:172, 2000; AJHG 71-suppl4: abs.2236, 2002). In order to identify variants that could be used as anthropogenetic markers and to verify the classification of the 21 non CF-causing mutations found in the European population, we enlarged this analysis to a sample of 59 individuals from Burkina Faso (Africa). All the exons plus the intronic flanking regions of the CFTR gene were screened by DGGE and sequencing. Ten variants were found: 94C/T, R74W, IVS6a-GATT, M470V, 1716G/A, 2694T/G, 3030G/A, 3041-71G/C, 4002A/G, 4521G/A. Moreover, an anomalous DGGE pattern not yet characterized by sequencing was found in 2/31 subjects in exon 14b. 94C/T was only found in the African population. The other mutations were found in the European population too. Four variants showed a frequency very different in the two populations, therefore they can be considered good anthropogenetic markers: 94C/T found in 4/112 African genes and absent in 924 European genes; M470V found with a frequency of 0.027 in the African genes compared with 0.618 in the European genes; 4002A/G found with a frequency of 0.172 in African and of 0.036 in European population; and the not yet characterized variant of exon 14b, found in 2/62 African genes and absent in the European genes.

**Frequencies of normal and disease SMN1 alleles, SMN1-SMN2 haplotypes, and de novo SMN1 mutations: implications in SMA genetic risk assessment and molecular evolution of SMN.** *S. Ogino*<sup>1, 2, 3</sup>. 1) Pathology, Brigham and Women's Hospital, Boston, MA; 2) Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; 3) Harvard Medical School, Boston, MA.

Approximately 94% of clinically typical spinal muscular atrophy (SMA) patients lack both copies of SMN1 by gene deletions/conversions (0-copy alleles). Genetics of SMA is complex because of the presence of small intragenic SMN1 mutations (1D alleles), two copies of SMN1 on one chromosome 5 (normal 2-copy allele), a high de novo mutation rate, and the SMA modifier gene SMN2 that is highly homologous to SMN1. Because of the genetic complexity and the high carrier frequency (~1/50), genetic counseling and risk assessment are very important. However, previous studies on SMN1 allele frequencies were based on relatively limited amount of data. For the most accurate genetic risk assessments, using all available and reliable data, I calculated various normal and disease SMN1 allele frequencies as follows: 0-copy, 0.0099; normal 1-copy, 0.95; 2-copy, 0.043; and 1D, 0.00018. The paternal and maternal de novo mutation rates were  $2.1 \times 10^{-4}$  and  $4.2 \times 10^{-5}$ , respectively. For the better understanding of molecular evolution of the SMN genes, I also estimated frequencies of various SMN1-SMN2 haplotypes [designated as (SMN1 copy number)-(SMN2 copy number)] as follows: 0-0, 0.00037; 0-1, 0.0066; 0-2, 0.0032; 1-0, 0.26; 1-1, 0.67; 1-2, 0.013; 2-0, 0.033; and 2-1, 0.010. In conclusion, the calculated SMN1 allele frequencies and de novo mutation rates can be utilized for accurate genetic risk assessments. The relatively high frequency of the 0-2 haplotype among disease haplotypes, and of de novo conversion mutations, implies that nucleotide position 840 (the location of an essential difference between SMN1 and SMN2) may constitute a mutation hotspot. The presence of rare 3-copy-SMN1 alleles was also suggested.

**Linkage disequilibrium and polymorphism of HLA genes in the Shona of Zimbabwe.** *D. Meyer*<sup>1</sup>, *K.A. Mather*<sup>1</sup>, *R. Jackman*<sup>2</sup>, *C. Yiu*<sup>2</sup>, *M. Sohsman*<sup>2</sup>, *V. Nguyen*<sup>2</sup>, *D. Katzenstein*<sup>3</sup>, *P. Mason*<sup>4</sup>, *G. Thomson*<sup>1</sup>, *L. Louie*<sup>2</sup>. 1) Integrative Biology, UC Berkeley, Berkeley, CA; 2) CHORI, Oakland, CA; 3) Stanford University Medical School, CA; 4) U of Zimbabwe Medical School, Zimbabwe.

Genetic studies of African populations have established that they present high levels of genetic diversity, relative low levels of linkage disequilibrium, and harbor variation that gave rise to that seen in non-African populations. These patterns are usually interpreted to reflect the demographic history of African populations, which are likely to have experienced larger effective population sizes and have older population histories. In this study, we use a sample of 229 Shona individuals from Zimbabwe to investigate whether these features hold for a set of genes that are known to be experiencing natural selection, the HLA classical genes. Heterozygosity levels at HLA genes range from 0.79 for HLA-DQA to 0.94 for HLA-B. These heterozygosities are higher than nuclear non-HLA genes in human populations (Stephens et al. 2002), and are among the highest HLA heterozygosity values among worldwide populations. All 3 loci within the class I region (1.6Mb) are in significant LD with each other. Excluding DPB1 (which is not in significant LD with any other locus), all class II loci (spanning 75kb) are also in significant pairwise LD. Spanning class I and II (distances of ~1.2Mb), a few pairs of loci are in significant LD: B with DRB1, DQA1 and DQB1, and C with DRB1, demonstrating that LD in the Shona exists over distances longer than the size of haplotype blocks. The CEPH (Sanchez-Mazas et al., 2000) and three Oaxacan Amerindian populations (Hollenbach et al., 2001) shows similar patterns of pairwise LD. However, the strength of LD is higher in the Oaxacan populations than either the Shona or CEPH, consistent with the expectation of higher LD in small constant sized populations than those that have expanded. This comparison of an African to non-African populations shows that HLA gene LD and diversity are shaped by demographic history in a similar manner to the remainder of the genom.

**Linkage Disequilibrium in the CEPH, Yoruba, Southern Han and Mixe populations in six genomic regions.** *K.A. Mather*<sup>1</sup>, *M. President*<sup>1</sup>, *S. Easton*<sup>2</sup>, *J. Hollenbach*<sup>1</sup>, *W. Klitz*<sup>3</sup>, *G. Huttley*<sup>2</sup>, *G. Thomson*<sup>1</sup>. 1) Integrative Biology, Univ California, Berkeley, Berkeley, CA; 2) Australian National Univ., Canberra, Australia; 3) CHORI, 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 exceeded that found in the HLA (Human Leukocyte Antigen) region (Huttley et al. 1999). The high LD regions were hypothesized to be experiencing selection in the CEPH. However, if selection pressures vary among populations, patterns of LD may differ between populations. In order to determine if the LD pattern is conserved across populations, we analyzed microsatellite LD. We examined six genomic regions (5 - 10 Mb in size) on chromosomes 6 (the HLA region), 3 and 20 (two control region without high LD in the CEPH), 7 - one region on each arm and 22 (three CEPH high LD regions) in four populations: Mixe Amerindians (n=49), Southern Han Chinese (n = 47), CEPH (n = 49) and Yoruba Africans (n = 50). We quantified LD by the proportion of marker pairs with  $D'$  exceeding the 90th percentile of  $D'$  between unlinked markers in that population. 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, Gabriel et al. 2002). Across all regions, the Mixe population have the highest LD levels, followed by the southern Han and CEPH populations and the Yoruba have the lowest LD, consistent with the theoretical expectation (Slatkin 1994) that small populations will have more LD than large populations which have expanded. The ordering of five regions by amount of LD is similar in all populations (but distinct from that of the earlier CEPH study): 6>3>22>20>7p, suggesting that LD levels in these regions may reflect a similar ordering in selection intensities across populations. The LD levels of the region on chromosome 7q vary between populations, suggesting that selection intensities in this region vary between populations.

**Evolution of Class I ADH haplotypes in worldwide human populations.** *Y. Han, M.V. Osier, A.J. Pakstis, H. Oota, J.R. Kidd, K.K. Kidd.* Genetics, Yale University, New Haven, CT.

The seven human Alcohol dehydrogenase (ADH) genes code for a family of enzymes that catalyze the reversible oxidation of alcohols to aldehydes. The three Class I ADH genes (ADH1A, ADH1B, ADH1C) share 95% sequence identity and are believed to play a major role in ethanol metabolism. Variants of different Class I ADH genes are associated with a protective effect against alcoholism. We have typed nine Class I ADH SNPs (ADH1C EcoR I, HaeIII, Arg271Gln, Ile349Val, Pro351Thr, ADH1B Arg47His, RsaI, Arg369Cys, ADH1A BccI) spanning > 68kb of the > 77kb Class I cluster, in samples of 38 populations. Most of the haplotypes that are common in Sub-Saharan Africa can be accounted for by a simple multibranching tree of nucleotide substitutions starting from the rare ancestral haplotype. The most common haplotype in the world differs from the ancestral haplotype by two nucleotide substitutions; the intermediate differing from the ancestor at only one of those sites is rare but present in African populations. The second most common haplotype differs from the most common haplotype at two additional changes; again, the intermediate differing at only one of those sites is found only in Africa. Thus, the haplotype frequencies suggest that most haplotypes were generated in Africa before modern humans migrated out of Africa. The haplotype that harbors a functional substitution associated with protection against alcoholism (ADH1B\*47His) is seen exclusively and at high frequency (>60%) in East Asia and appears to be a crossover product involving two haplotypes common in Western Asia. This crossover haplotype is not found in indigenous Africans suggesting it arose from a recombination event among modern humans who had already left Africa. Regionally restricted functional variants also exist in Africa and the Americas suggesting selection is operating on these genes probably related to adaptation in each geographical region. [Supported in part by NIH grant AA09379].

**Gender-specific Association Between *IL10* Polymorphisms and the Rate of Decline of Lung Function in Smokers.**

*J-Q. He*<sup>1</sup>, *JE. Connett*<sup>2</sup>, *NR. Anthonisen*<sup>3</sup>, *PD. Paré*<sup>1</sup>, *AJ. Sandford*<sup>1</sup>. 1) Medicine/McDonald Research Lab, University of British Columbia, Vancouver, BC, Canada; 2) Division of Biostatistics, School of Public Health, University of Minnesota; 3) Faculty of Medicine, University of Manitoba.

Interleukin-10 (IL10) is a cytokine that has a wide spectrum of anti-inflammatory actions. IL10 concentrations are reduced in induced sputum from patients with COPD, indicating that this might be a mechanism for increasing lung inflammation. Several single nucleotide polymorphisms (SNPs) in the *IL10* promoter have been associated with the level of expression of IL10. Objective: To determine whether allelic variants of the *IL10* gene are associated with rate of decline of lung function in smokers. Methods: We used a case-control approach to study 585 Caucasians with the fastest decline in forced expiratory volume in 1 second (FEV<sub>1</sub>) (n=280, FEV<sub>1</sub> = -153 3ml/year) and with the slowest FEV<sub>1</sub> decline (n=305, FEV<sub>1</sub> = +15 3ml/year) selected from among 3,216 subjects who continued to smoke during 5 years of follow up in the NHLBI Lung Health Study. Genotyping was performed by TaqMan assay. The associations were analyzed by logistic regression to adjust for potential confounding factors. Results: We chose 3 SNPs from more than 20 SNPs of the *IL10* gene because of a minor allele frequency greater than 10% and because these 3 SNPs were able to define more than 80% of all haplotypes in the *IL10* gene. They were *IL10* -627 C/A (or -592 C/A), 734 T/G (perfect linkage disequilibrium with -1082 A/G) and 3368 G/A. Neither the genotype or haplotype frequencies were significantly different between groups with the fastest and slowest decline of lung function when the whole group was analyzed, however women who were homozygous for the 3368G allele were more likely to be in the fast decline group (70.3% vs 58.1% OR = 2.3, p = 0.02). To date there are no data testing whether the 3368 A/G transition has functional significance. Conclusion: Genetic polymorphisms in the *IL10* gene were associated with the rate of decline of lung function in woman smokers. Supported by the NHLBI and the CIHR.

**Mongolian populations as genetic resource: Challenges towards genetic study in Mongolians.** *B. Munkhbat<sup>1</sup>, K. Tounai<sup>1</sup>, T. Katoh<sup>1</sup>, S. Mano<sup>1</sup>, H. Ando<sup>1</sup>, S. Ando<sup>1</sup>, N. Baasanjav<sup>2</sup>, G. Batmunkh<sup>3</sup>, H. Inoko<sup>1</sup>, G. Tamiya<sup>1</sup>, N. Munkhtuvshin<sup>2</sup>.* 1) Molecular Life Science, School of Medicine, Tokai University, Isehara, Kanagawa, Japan; 2) Central Scientific Research Laboratory, National Institute of Medicine, Ulaanbaatar, Mongolia; 3) Department of Physiology, National Medical University of Mongolia, Ulaanbaatar, Mongolia.

To select the subject populations is a crucial factor for the genetic studies of the identification of genomic regions or genes predisposing to complex diseases. Because of reduced genetic and environmental variability, the genetic isolates have been thought to facilitate initial gene mapping of both complex and monogenic diseases. On the other hand, general outbred population better suit for the fine mapping of the complex and monogenic diseases. The National Institute of Medicine, Mongolia (NIMM) has started a scientific project on comprehensive genome wide analysis in contemporary Mongolians in collaboration with Tokai University, Japan. The project concentrates on cataloging DNA/cell-line samples from Mongolian populations and then disease association/linkage studies using these samples. Specific features of the Mongolians such as small population size (2.4 million people by 2000 population census), existence of nearly 20 closely related as well as genetically distinct ethnic groups in the vast territory (1.5 million km<sup>2</sup>), large families, cooperative people, and remote and centralized medical service make it an ideal population for the genetic studies of various purposes and consequently would offer an attractive scientific outcome. While presenting the genetic features of the Mongolian ethnic groups on the basis of a pilot study using STR and SNP markers, we will describe our project concept and also our efforts to attract potential corporate partners worldwide.

**SNP variation in a non-coding region of Xq13.3 in East Asia.** *T. Katoh<sup>1</sup>, K. Tounai<sup>1</sup>, S. Mano<sup>1</sup>, H. Ando<sup>1</sup>, B. Munkhbat<sup>1</sup>, N. Munkhtuvshin<sup>2</sup>, K. Tokunaga<sup>3</sup>, G. Jia<sup>4</sup>, H. Inoko<sup>1</sup>, G. Tamiya<sup>1</sup>.* 1) Molecular Life Science, School of Medicine, Tokai University, Isehara, Kanagawa, Japan; 2) Central Scientific Research Laboratory, National Institute of Medicine, Ulaanbaatar, Mongolia; 3) Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 4) Harbin Red Cross Blood Center, Harbin, China.

It is well known that East Asia consists of various human populations. However, the genetic relationships among these populations are still unclear and an interesting subject in human genetics. To study the relationship among some East Asian populations including Chinese, Japanese, Korean, and Mongolian, we analyzed the SNP variations found within 10kb length at a non-coding region of Xq13.3. This region is thought to have an extremely low level of recombination rate, which is approximately one-eighth of the average of X chromosome, implying that it will be useful for evolutionary analyses. Frequency distributions of the Xq13.3 haplotypes showed that East Asian populations have the relatively low level of diversity than non-Asian populations, such as European American and African American, suggesting an occurrence of past bottleneck event in East Asia. On the whole, our study is compatible with the previous studies using Y chromosome, although somewhat different patterns are observed between Y chromosome and our studies.

**SNP-haplotype analysis in human chromosome region 3q27 in Japanese and European Americans.** *H. Morisaki*<sup>1</sup>, *E. Inoue*<sup>2</sup>, *Y. Kitamura*<sup>2</sup>, *N. Kamatani*<sup>2</sup>, *T. Morisaki*<sup>1,3</sup>. 1) Dept Bioscience, NCVI Research Inst, Suita Osaka, Osaka, Japan; 2) Inst Rheumatol, Tokyo Womens Med Univ; 3) Dept Mol Pathophysiol, Osaka Univ Grad Sch Pharm Sci.

SNP-haplotype analysis, along with the identification of haplotype-tagging SNPs, is believed to be a powerful tool to study genetic association with common diseases. We analyzed genetic variation and haplotype structure of human chromosome region 3q27, which has been shown to be associated with type 2 diabetes in several populations including Japanese. Fifty-six marker SNPs, spanning 1 Mb region of chromosome 3q27, were selected and genotyped in 94 Japanese control subjects and 95 European American subjects. To define haplotype blocks, we used  $D'$  confidence bounds instead of point estimates applying the same criteria defined by Gabriel, et. al. Among 56 marker SNPs, 53 SNPs were common (>10%) and shared between both populations, and more than 95% of SNP pairs were informative in terms of historical recombination. Ten and twelve haplotype blocks were defined in Japanese and European Americans, respectively. Six of them shared their boundaries and none of them were discordant between populations. The minimal span of the blocks varied from 2 kb to 51 kb, and the block-boundaries were not necessarily concordant with the gene-boundaries. Each haplotype block was consisted of 2 to 5 common (>5%) haplotypes. Most of them were shared in both populations, but differed in the haplotype constitution, resulting in different pairwise SNP correlation ( $r^2$ ) between populations. Haplotype-tagging SNPs were identified in two-thirds of the cases, which differ slightly between populations. We also found that the two SNPs in APM1 gene, one in promoter region reported to be associated with diabetes in French subjects, and the other in intron 2 which showed association in Japanese patients, were assigned to different haplotype blocks with the evidence of historical recombination between them. It may suggest a different mechanism of APM1 gene function affecting the diabetic phenotype.

**Strategy for a systematic genomic analysis of the Mexican population.** *G. Jimenez-Sanchez<sup>1,2,3</sup>, J.P. Lacleste<sup>1,4</sup>, J. Rosenkranz<sup>1</sup>, A. Serrano-Perez-Grovas<sup>1,5</sup>, M. Uribe<sup>1,6</sup>.* 1) Consortium Institute of Genomic Medicine, Mexico City, Mexico; 2) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore MD, USA; 3) Mexican Health Foundation (FUNSALUD), Mexico; 4) National Autonomous University of Mexico, Mexico; 5) National Council of Science and Technology (CONACYT), Mexico; 6) Ministry of Health (SSA), Mexico.

Mexico prepares a nationwide platform to develop genomic medicine focused on medical problems of its population. One of the initial goals is to study the genomic structure of the Mexican population, with special interest in areas of the human genome directly related to health problems. Unlike any other population, Mexicans mostly result from the mixture of over 65 native Indian groups that arrived in Mesoamerica during the last 15 centuries, with Spaniards that arrived in Mexico about 500 years ago. Since 1999, over 100 biomedical investigators from different backgrounds in Mexico and abroad have joined efforts with government, academia and industry to identify priorities and goals for genomic medicine in Mexico. We recently reported the Mexican strategy to develop a nationwide platform on genomic medicine (*Science* 300; 5617, 2003). We have defined a five-year plan to achieve such goals, by developing a Mexican Institute of Genomic Medicine with strong intramural and extramural programs for basic and clinical research, professional and public education, and technology. Our feasibility study estimated an investment of \$190 Million USD in infrastructure and operations for the first five years. Initial candidate populations for genomic analysis have been identified and ethical, legal and social issues, along with technical requirements are being evaluated. This plan intends to serve as the bases for a nationwide platform in which genomic medicine can impact on national and regional health problems. Furthermore, we predict that the systematic genomic analysis of the Mexican population will have significant medical and financial benefits not only in Mexico, but also in the United States where Mexicans represent a significant part of the population. Detailed information is available at [www.inmegen.org.mx](http://www.inmegen.org.mx).

**Polymorphisms of five hypervariable autosomal loci in different ethnic groups of Eastern Europe. D.A.**

*Verbenko<sup>1</sup>, P.A. Slominsky<sup>1</sup>, E.K. Khusnutdinova<sup>2</sup>, V.A. Spitsyn<sup>3</sup>, N.A. Bebyakova<sup>4</sup>, A.I. Mikulich<sup>5</sup>, S.A. Limborska<sup>1</sup>.* 1) Human Molecular Genetics Department, Institute of Molecular Genetics, Moscow, Russia; 2) Institute of Biochemistry and Genetics, Ural Branch of Russian Academy of Sciences, Ufa, Russia; 3) Medical and Genetics Scientific Centre, Russian Academy of Medical Sciences, Moscow, Russia; 4) North State Medical University, Arkhangelsk, Russia; 5) Institute of Art History, Ethnography and Folklore, National Academy of The Republic of Belarus, Minsk, Belarus.

A study of DNA diversity is very important to understand the nature of human populations relationships. Here we present our results of East European ethnic groups relationships based on autosomal DNA markers with tandem repeat structure. These markers are extremely polymorphic and are widely used in medical and population genetics.

Eastern European region inhabited by a number of ethnic groups belongs both to two main anthropological types and to their admixtures. We considered Eastern Slavs as Europeans, whereas Kalmyks and Yakuts as Asians. Ural region ethnic groups (Komis, Mordvinians, Bashkirs, Udmurts) were regarded as admixture populations with different levels of an Asian component.

The analysis of DNA minisatellite polymorphisms (3'ApoB and D1S80 loci) was carried out using the PCR and subsequent electrophoresis followed by silver staining. The triplet microsatellites (DM, DRPLA, SCA1 loci) variability was investigated using 33P-labelling of PCR primers. All of the loci studied shown high polymorphism level. Genetic distance analysis was based on multidimensional scaling of Nei's pairwise genetic distance matrix. An UPGMA phylogenetic tree was also constructed. Observed cluster patterns reveal maximum distance between Europeans and Asians, while Uralic populations are most closely related to Europeans. As a part of the conclusion, we can strike very close relationship between Eastern Slavonic populations, namely Russians, Byelorussians, and Ukrainians. The data obtained are in agreement with ethno-historic and linguistic studies of the Eastern European region.

**Smoking-dependent effects of the Angiotensin-Converting Enzyme gene insertion/deletion polymorphism on blood pressure.** *A.F.C. Schut<sup>1,2</sup>, F.A. Sayed-Tabatabaei<sup>1</sup>, J.C.M. Witteman<sup>4</sup>, A.M. Bertoli Avella<sup>1</sup>, J.M. Vergeer<sup>1</sup>, H.A.P. Pols<sup>2,4</sup>, A. Hofman<sup>4</sup>, J. Deinum<sup>3</sup>, C.M. van Duijn<sup>1</sup>.* 1) Genetic Epidemiology, Erasmus MC, Rotterdam, The Netherlands; 2) Internal Medicine, Erasmus MC, Rotterdam, The Netherlands; 3) Internal Medicine, UMC Radboud, Nijmegen, The Netherlands; 4) Epidemiology & Biostatistics, Erasmus MC, Rotterdam, The Netherlands.

Studies on the role of the Angiotensin-Converting-Enzyme (ACE) gene in the development of hypertension have yielded conflicting results. Recent studies suggested that the ACE gene may have smoking-dependent effects on the development of cardiovascular disease. We studied the relation between the ACE insertion/deletion polymorphism, blood pressure and risk of hypertension in current, former and non-smokers in a population-based cohort.

We included 2414 non-smokers, 2794 former smokers and 1508 current smokers, all participants of the Rotterdam Study. In each group, we assessed the relation between the ACE I/D polymorphism, systolic (SBP) and diastolic blood pressure (DBP) and risk of hypertension. Mean blood pressure levels and prevalence of hypertension were compared between carriers and non-carriers of the D-allele. All analyses were adjusted for age, sex, BMI, diabetes mellitus, HDL-cholesterol, total cholesterol and anti-hypertensive medication use.

In non- and former smokers, blood pressure and the risk of hypertension did not differ significantly between genotypes. In smokers, we found a significant increase in SBP in DD-carriers (139.6 22.8 mmHg) compared to II-carriers (136.0 22.7 mmHg)( $p=0.04$ ). No effect of ACE genotype was observed for DBP. The risk of hypertension was significantly increased in smokers who carried one (OR: 1.4; 95% CI: 1.0-1.9;  $p=0.05$ ) or two copies of the D-allele (OR: 1.5; 95% CI: 1.1-2.2;  $p=0.02$ ).

The D-allele of the ACE polymorphism is associated with a modestly, but significantly, increased SBP and risk of hypertension in smokers. Our study underlines the importance of gene-environment interactions in the study of candidate genes for hypertension.

**The European ACP1\*C Allele has Recessive Deleterious Effects on Early Life Viability.** *J. Wilder, M. Hammer.*  
Division of Biotechnology, University of Arizona, Tucson, AZ.

The acid phosphatase locus (ACP1) is a classical polymorphism that has been surveyed in hundreds of human populations world-wide. Among individuals of European ancestry, the ACP1\*C allele occurs with an average frequency of 0.05, while it is nearly absent in all other human populations. It has been hypothesized that this allele is maintained by overdominant selection among European populations. Here we analyze ACP1 protein polymorphism data from over 46,000 individuals previously surveyed in 66 populations across Europe, as well as inheritance data from over 6000 European parent offspring pairings to assess the signature of natural selection on this allele. Although we see a significant excess of ACP1\*C heterozygotes relative to Hardy-Weinberg expectations, we find no evidence that natural selection favors ACP1\*C heterozygotes. Instead, ACP1\*C appears to have a strongly deleterious and recessive fitness effect, leading to only 48.6% of expected homozygous offspring from heterozygous parents, and significantly fewer homozygotes than expected among populations. Because parent offspring pairings indicate a significant deficiency of ACP1\*C homozygotes, we infer that viability selection is acting on ACP1\*C homozygotes very early in life, perhaps before birth. We estimate that approximately 1.1% of all couples of European ancestry are composed of individuals who both carry the ACP1\*C allele. As such, selection against ACP1\*C homozygotes may represent a non-negligible contribution to the overall number of spontaneous abortions among women of European ancestry, and may cause substantial fertility reductions among some combinations of parental genotypes.

**Angiotensin converting enzyme gene is associated with Alzheimers disease in women.** *K. Sleegers<sup>1</sup>, T. Den Heijer<sup>1,2</sup>, E.J. Van Dijk<sup>1,2</sup>, A. Hofman<sup>1</sup>, P.J. Koudstaal<sup>2</sup>, M.M.B. Breteler<sup>1</sup>, C.M. Van Duijn<sup>1</sup>.* 1) Epidemiology&Biostatistics, Erasmus MC, Rotterdam, The Netherlands; 2) Department of Neurology, Erasmus MC, Rotterdam, The Netherlands.

Despite strong evidence for a role of Angiotensin converting enzyme (ACE) in Alzheimers disease (AD), studies assessing the relation between the ACE I/D polymorphism and AD show conflicting results. A problem in the interpretation of findings is lack of power (Lohmueller et al, Nature Genetics 2003). Moreover, since ACE plays a pivotal role in vascular homeostasis, omitting cardiovascular status as a possible intermediate or confounder may lead to false associations. We evaluated the association between ACE and AD in a large population based cohort study (Rotterdam Study, n=6869) adjusting for confounding by cardiovascular risk. In addition we performed a meta-analysis of all studies conducted up to December 2002 including our data (3749 cases, 10495 controls). In the Rotterdam Study we further explored the underlying pathophysiology by analyzing the relation between ACE and early AD-related pathology on MRI, i.e. hippocampal and amygdalar atrophy, white matter lesions and infarcts, in 494 non-demented elderly. In the meta-analysis we found a modest but significantly increased prevalence of the I-allele in patients (OR 1.15 (95%CI 1.01-1.30)). In the Rotterdam Study, during 6 years of follow-up, subjects homozygous for the I-allele had a modestly increased risk of AD of 1.12 (95%CI 0.99-1.25; p=0.06) compared to subjects with a D-allele. This finding was strongest in women. After adjustment for cardiovascular confounders female carriers of the II genotype had a 1.39 fold increased risk of AD (p=0.001). Likewise women with the II genotype had lower hippocampal (p=0.02) and amygdalar (p=0.05) volumes on MRI. Vascular pathology on MRI was not associated with ACE. In conclusion the results of the meta-analysis, the follow-up study of AD and the study of early pathology on MRI consistently show an association of the ACE I-allele with AD in women independent of cardiovascular disease. Our findings underscore the value of large-scale epidemiological follow-up of (early) pathology for genetic research.

**Global distribution of the PRNP codon 129 polymorphism suggests an increased risk of CJD and vCJD in East Asia.** *M. Wolujewicz, R. Kaushal, A. Bhati, G. Sun, R. DeKa.* Environmental Health, University of Cincinnati, Cincinnati, OH.

The codon 129 polymorphism of the prion protein (PRNP) gene is characterized by an amino acid substitution of valine for methionine. Homozygosity at this locus has been described as influencing susceptibility to prion diseases, namely Creutzfeldt-Jakob disease (CJD). Additionally, individuals affected with vCJD, the variant form of the disease, understood to be transmitted by the bovine spongiform encephalopathy (BSE) epidemic, have all been identified as homozygous for methionine at this locus (Collinge et al. 1996, Lancet 348). The distribution of the polymorphism in European populations has been shown to be 40% Met/Met, 50% Met/Val, and 10% Val/Val. We examined the PRNP 129 polymorphism in 565 individuals comprising 15 global populations distributed over Africa, Europe, Asia, and Oceania. The populations of East Asia have significantly lower heterozygosity with an increased distribution of Met homozygosity (0.86-0.96), suggesting an increased risk for developing CJD in these populations. Also, recent reports of BSE in Japan (2001 Nature, 413) introduces the possibility for another vCJD epidemic, now with especial concern because of the Met/Met genotype frequency in the area. Projections of vCJD risk in Japan and other East Asian countries need to incorporate the high frequency of this genotype to more accurately ascertain risk. (Supported in part by NIH grant ES06096).

**Inferring intraspecific genealogy: a stepwise partitioning approach.** *G. Zhang, L. Jin.* Center for Genome Information, Dept. Environmental Health, University of Cincinnati, Cincinnati, OH.

In a population, a chromosome is delimited, by historical recombination events, into smaller blocks (i.e. haplotype blocks), each of which is captured by a genealogy representing a unique evolutionary history of the block. An efficient and accurate inference of such genealogy or phylogeny is essential in (a) understanding the evolutionary history and genetic structure of the population, (b) unraveling important biological processes such as recombination and mutation, and (c) applying such information in mapping and identifying the mutations and haplotypes underlying diseases and other traits. It also bears particular practical importance in inferring haplotype blocks and identifying haplotype-tagging SNPs (htSNPs). Based on the idea that any phylogenetic relationships of a DNA fragment without observable recombination can be represented by a primarily bifurcating tree with, to a less degree, multifurcating structure, we developed a stepwise partitioning algorithm for inferring such genealogy. In this method, all the sequences of a tree are partitioned (carrying one or a few specific mutations) by all the variations in a consecutive manner, and a unique nested partition can be obtained. And the final tree topology is solved by favoring the assumption that the number of mutations is proportional to the coalescent time. We have applied this method to simulated data sets and obtained reliable results that are superior to the existing maximum parsimony methods. Another major strength of this approach is its efficiency, much faster than any of the existing tree-making methods.

**HLA haplotyping in ethnic communities of Kerala, South India.** *R. Thomas, S.B. Nair, M. Banerjee.* Human Molecular Genetics Lab, Rajiv Gandhi Centre for Biotechnology, Thycaud P.O., Trivandrum, Kerala, INDIA.

Human Leukocyte Antigens (HLA) are glyco-proteins expressed on the surface of every cell in the body. The molecules form part of a system of immune recognition to distinguish "self" from "non-self". Their role is to present peptides, derived from foreign antigens, to cytotoxic T cells and to enable the specific destruction of "non-self" cells. Variations in these peptides between different individuals alter the nature of the antigen presented to the immune system. These antigenic variations could give a clinical advantage over some infectious diseases. It can also prove to be disadvantageous for some immune response diseases.

India has the worlds highest ethnic diversity with a hierarchical caste structure. The basis of reproductive isolation of these populations is also highly varied, comprising of geographic, religious, ethnic or occupational backgrounds. In the present study we selected eight ethnic communities namely Malapandaram, Paniya, Adiya, Kurichiya, Kanikkar, Kattunaikkar, Kuruma and Pulaya and one group consisting of individuals from the mainstream population of Kerala. These population groups are of Dravidian origin and are believed to be the earliest settlers of Southern India. We identified the distribution of HLA-B and HLA-C alleles in these communities using Polymerase Chain Reaction-Sequence Specific Primer (PCR-SSP) method based on the IHWC protocol. A total of 18 alleles HLA-B alleles were identified in the mainstream population while only 5-10 alleles were observed in the eight ethnic communities. B\*7, B\*44, B\*51 and B\*61 were the most common alleles observed. A total of 13 HLA-C alleles were identified with Cw\*07, Cw\*04, Cw\*14 and Cw\*15 occurring at a high frequency. It is interesting to note that Cw\*08 which was reported to be a characteristic feature of Indians of Indo European origin was present at a very low frequency in the present study which mainly comprises populations of Dravidian origin. Cw\*15 is believed to be of Dravidian origin as it was absent in Indians of Indo-European origin. The two-point haplotypes occurring at a high frequency were B\*07-Cw\*07, B\*35-Cw\*07, B\*44-Cw\*07, B\*58-Cw\*10, B\*61-Cw\*10 and B\*62-Cw\*14.

**Founder Mutations among the Dutch.** *M. Zeegers*<sup>1</sup>, *R. Vlietinck*<sup>1</sup>, *F. van Poppel*<sup>2</sup>, *L. Spruijt*<sup>1</sup>, *H. Ostrer*<sup>3</sup>. 1) Maastricht Univ, Maastricht, The Netherlands; 2) Netherlands Interdisciplinary Demographic Institute, The Hague, The Netherlands; 3) NYU School of Medicine, New York, NY.

Many genetic disorders demonstrate mutations that can be traced to a founder, sometimes a person who can be identified. These founder mutations have generated considerable interest, because they facilitate studies of prevalence and penetrance and can be used to quantify the degree of homogeneity within a population. This study reports on founder mutations among the Dutch and relates their occurrence to the history and demography of the Netherlands. International migration, regional and religious endogamy, and rapid population growth played key roles in shaping the Dutch population, a country that now includes 16 million inhabitants. Several different classes of founder mutations have been identified among the Dutch. Some mutations occur among people who represent genetic isolates within this country. These include mutations for benign familial cholestasis, diabetes mellitus, type I, infantile neuronal ceroid lipofuscinosis, L-DOPA responsive dystonia and triphalangeal thumb. Although, not related to a specific isolate, other founder mutations occur only within the Netherlands, including those predisposing for hereditary breast-ovarian cancer, familial hypercholesterolemia, frontotemporal dementia, hereditary paragangliomas, juvenile neuronal ceroid lipofuscinosis, malignant melanoma, protein C deficiency and San Filippo disease. Many of these show a regional distribution, suggesting dissemination from a founder. Some mutations that occur among the Dutch are shared with other European populations and others have been transmitted by Dutch migrants to their descendants in North America and South Africa. The occurrence of relatively limited genetic heterogeneity for many genetic conditions among the Dutch has resulted in short chromosomal regions that have remained identical by descent. These observations demonstrate the opportunity for gene discovery for other diseases and traits in the Netherlands.

**Meta-analysis of the evidence for sex-differential fitness of BRCA2 variant.** *D. Teare, A. Cox, C. Anderson, J. Shorto, C. Cannings.* Division of Genomic Medicine, University of Sheffield, Sheffield, South Yorks, UK.

Healey et al (2000) reported that a common variant (N372H) in exon 10 of the BRCA2 gene may affect foetal viability in a sex dependent manner. Their results suggested that females (adults and newborns) generally showed a similar excess of heterozygotes, whereas the single large sample of newborn males demonstrated decreased heterozygote fitness. Their study did not include adult males. While the original analysis reported the sex-specific fitness estimated under the assumption of equilibrium, no attempt was made to evaluate whether this equilibrium was stable.

In order to study the possible sex differential effect in more detail we have genotyped further series of adult males and females. Recent publications provided two further samples of female adult controls. A joint analysis of all 11 available female samples supports the original observation for female heterozygote advantage. However the evidence for reduced fitness in male heterozygotes is now less clear. In addition to this meta-analysis we have considered the range of stable sex-differential fitness models which would be consistent with observed data. The point estimates in the original publication were not consistent with a stable solution but the results of our presented meta-analysis are.

**GENOMEUTWIN - an integrated project of eight large twin cohorts for genetic studies on complex traits.** *L. Peltonen, M. Perola, D. Boomsma, K. Christensen, A. Evans, J. Harris, H. van Houwelingen, J. Kaprio, K. Kuulasmaa, K. Ohm Kyvik, J.E. Litton, N. Martin, N. Pedersen, T. Spector, A. Stazi, A.C. Syvanen.* National Public Health Inst, Finland.

Large population-based cohorts are of special significance for genomic research aiming to characterize the background of common human traits and diseases. European twin cohorts provide a unique opportunity for investigation of the role of genetics and environment/life style in the etiology of common traits. The EU-funded integrated project GenomeUtwinn ([www.genomeutwin.org](http://www.genomeutwin.org)) pools the expertise of eight twin cohorts (Australian, Danish, Dutch, Finnish, Italian, Norwegian, UK, Swedish) and a large European prospective epidemiological study of cardiovascular disease: MORGAM ([www.ktl.fi/morgam](http://www.ktl.fi/morgam)). The study population includes over 600.000 twin pairs and over 130.000 participants in MORGAM cohorts. So far over 200.000 biological samples have been collected for DNA analyses and major effort invested on the harmonization of database systems and phenotypic criteria. The GenomeUtwinn applies and develops new statistical strategies to utilise the unique, longitudinal information of health and life events of study participants to characterise the etiology of common traits and diseases, initially obesity, stature, longevity, migraine, coronary heart disease and stroke. The GenomeUtwinn operates via five intellectual cores on epidemiology, database, genotyping, statistics and ethics, which provide the infrastructure and expertise for the project. So far the twin cohorts have been mined for informative pairs and core families for ongoing genome scans. Putative loci are being fine mapped and SNP haplotypes constructed for the regional genes to determine the trait-associated alleles to be monitored in the prospective study cohorts to assess their contribution to survival and genetic risk. We have estimated heritability and the character of genetic component for traits listed above across the study populations. Examples of the data include the average heritability of 46 % for migraine in 31 410 twin pairs and evidence for different genetic effect of variations on BMI for males and females observed in 37 000 twin pairs.

**G Protein 3 Subunit (GNB3) C825T variation is correlated with latitude and climate: Is selection a source of differential susceptibility to hypertension?** *J.H. Young<sup>1</sup>, Y.-P. Chang<sup>2</sup>, J.-P. Chretien<sup>1</sup>, M. Ikeda<sup>2</sup>, J. Kim<sup>2</sup>, M.J. Klag<sup>1</sup>, M.A. Levine<sup>3</sup>, A. Chakravarti<sup>2</sup>.* 1) Dept of Medicine & Epidemiology, Johns Hopkins, Baltimore, MD; 2) Institute of Genetic Medicine, Johns Hopkins, Baltimore, MD; 3) The Children's Hospital, The Cleveland Clinic, Cleveland, OH.

Climatologic factors influence blood pressure and may have driven the evolution of genes involved in hypertension susceptibility. To test this hypothesis, we determined the extent to which latitude and climatologic factors contribute to worldwide variation in the GNB3 C825T, a functional polymorphism associated with hypertension. In the CEPH diversity panel, which includes 1064 people from 52 populations around the world, we genotyped C825T and five additional SNPs spanning a ~20 Kb region in and around GNB3. In order to compare variation in GNB3 versus the genome and assess the effect of demographic factors, over 400 microsatellite markers (STRs) were used to estimate genetic distance (Nei's D') among CEPH populations. Climate was characterized by average yearly temperature and precipitation. In univariate analysis, latitude explained 51% of C825T variation ( $\beta = -0.008$ ,  $R^2 = 0.5149$ ). Other GNB3 SNPs were only weakly associated with latitude (all  $|\beta| < 0.003$ , all  $R^2 < 0.0680$ ). No STR was as strongly correlated with latitude as C825T. In a separate model, temperature and precipitation together explained 38.9% of C825T variation ( $R^2 = 0.3893$ ). In the model containing latitude, temperature, and precipitation, each factor remained important ( $s$  were 0.010, -0.006, 0.002 respectively;  $R^2 = 0.5424$ ). Other GNB3 SNPs were also associated with climate but to a lesser degree (all  $R^2 < 0.1640$ ). Finally, C825T variation was not associated with relative genetic distance ( $R^2 = 0.003$ ). In addition, the inclusion of D' did not affect the association between C825T and latitude or climate. In summary, worldwide variation in C825T is largely explained by latitude and climatologic factors and shows a pattern distinct from the genome average. We speculate that differential exposure to selection pressures, most notably climate, during the out of Africa expansion contributed to GNB3 C825T variation and possibly differential susceptibility to hypertension.

Program Nr: 1332 from 2003 ASHG Annual Meeting

**Natural Selection on Haplotypes of MATP.** *J.C. Stephens, B.A. Salisbury, G. Ruano.* Population Genomics, Genaissance Pharmaceuticals, New Haven, CT.

We have used an empirical distribution of gene-based  $F_{ST}$  values to identify genes that may have been under intense natural selection in the course of modern human history. MATP, membrane associated transport protein, has the second highest  $F_{ST}$  in our analysis of thousands of genes. While our samples of African and Asian chromosomes are characterized by high levels of haplotypic diversity, the European sample is nearly fixed for a single, high  $F_{ST}$  haplotype. That haplotype is uniquely characterized by the derived amino acid Phe (Leu 374 Phe). The ancestral Leu allele is strictly conserved in all homologous proteins studied in other species, including the sucrose transporter in the celery plant. Had we only looked at a European population, we would have concluded that this gene is highly conserved and does not have much polymorphism. Furthermore, had we only looked at this one gene, we might have concluded that our European sample was too homogeneous, perhaps arising from a population bottleneck. The ability of this SNP to distinguish European from Asian and African ancestry has been noted before, but the haplotypic uniformity of European chromosomes containing this SNP is a novel finding illustrative of hitch-hiking at the haplotype level. This gene appears to affect melanin production or melanosome function according to studies in human, mouse, horse, and fish. Ultraviolet opacity of human skin is thought to be under strong selective pressure mediated by geographic variation in solar exposure and the contrasting pressures of the need for photo-induced vitamin D3 synthesis and the destructive effects of UV light exposure. If MATP's haplotypes are shown to affect human skin pigmentation, this gene's almost unrivalled selective signature would lend further support to these theories.

**Mitochondrial DNA Variation in Altaian Populations, the Putative Progenitors of Eurasian and Native American Populations.** *T.G. Schurr*<sup>1</sup>, *S.I. Zhadanov*<sup>1, 2</sup>, *L.P. Osipova*<sup>2</sup>. 1) Dept Anthropology, University of Pennsylvania, Philadelphia, PA; 2) Institute of Cytology and Genetics, Novosibirsk, Russia.

The Altai Mountain region has recently been suggested as the homeland for modern native Siberian and Native Americans whose common ancestors may have inhabited these lands many millennia ago. However, a number of distinct tribes that differ linguistically and anthropologically from each other now populate this region, with these differences probably reflecting their heterogeneous origins and ethnic histories. To ascertain the degree of biological relatedness of these groups, and determine the extent of their genetic contribution to the peopling of Eurasia and the Americas, we collected samples from several ethnic groups from the Altai Republic and analyzed sequence variation in their mtDNAs. These included 350 individuals from the northern Chelkans, Kumandinians, and Tubalars, and 275 individuals from the southern Altay-kizhi. For all mtDNAs, we sequenced the entire control region and screened each sample for diagnostic RFLP markers of West and East Eurasian haplogroups, while, for a subset of them, we also performed whole genome sequencing. Our initial results indicate that about 75% of Southern Altaian mtDNA haplotypes belong to East Eurasian maternal lineages, namely, haplogroups CZ, D, F, G, M8a, and M9a, while the remainder belong to West Eurasian haplogroups H, TJ, XI, and UK. Relatively similar frequencies of these haplogroups also occur in northern Altaians, who exhibit less diversity of West Eurasian haplogroups than southern Altaians. When contextualized with genealogical information, such data will allow us to reconstruct the genetic histories of Northern and Southern Altaian populations, as well as delineate the relationships between Altaian and other native Siberian populations, particularly other Turkic speaking groups such as Tunisiens, Tofalars and Yakuts, with whom they may share certain biological similarities.

**MALARIAL SELECTION AND THE DOGON: PATTERNS OF DNA SEQUENCE VARIATION AT THE -GLOBIN LOCUS.** *E.T. Wood<sup>1</sup>, D.A. Stover<sup>1</sup>, B.I. Strassman<sup>2</sup>, M.W. Nachman<sup>1</sup>, M.F. Hammer<sup>1</sup>*. 1) Ecol & Evol Biol, Univ Arizona, Tucson; 2) Anthropology, Univ Michigan, Ann Arbor.

Infectious diseases have affected humans throughout our evolutionary history yet the nature and magnitude of this selective pressure in shaping patterns of genetic variation remain elusive. To better understand the genetics of resistance to infectious diseases, we examine patterns of sequence variation at the -globin locus in the Dogon who reside in an endemic malarial environment in Mali, West Africa. The malarial resistance HbC allele is virtually restricted to West Africa and is found at a frequency of ~11% in the Dogon. We evaluated sequence variation in a number of contexts. First, we compare patterns of variation at -globin for 48 Dogon individuals to 3 other loci which are not likely to be under selection. Second, we compare patterns of variation at -globin in the Dogon with 5 other worldwide populations. Third, we resolve linkage phase across 1-kb located within the region of increased recombination for 38 HbAA, HbAC, and HbCC Dogon individuals. Fourth, we determine chromosomal linkage phase for 3-kb in the same region for 20 HbAC and HbCC individuals from Ghana, the Ivory Coast, and the Dogon. Neither levels of diversity (as measured by the HKA test) nor the skew in the frequency spectrum (as measured by Tajima's D) at -globin in the Dogon reject a model of neutral evolution. We also examine linkage disequilibrium patterns, which provides a more powerful approach for identifying selection on a new allele. The degree of linkage disequilibrium is compared between HbC (n=10) and HbA (n=66) chromosomes. We find that across a 1-kb region, only one HbC haplotype is represented; whereas, at least 6 haplotypes are found associated with the HbA chromosomes. An extended analysis of 3-kb also reveals fewer haplotypes associated with the HbC mutation than the HbA chromosomes. These results are consistent with a model of selection acting on the HbC alleles at the -globin locus in the Dogon. To further support this inference and to clarify the model of selection acting at -globin, we are extending the analysis to include more HbC chromosomes across a larger sequenced region.

**Family history, SELP and ITGB3 polymorphisms and their association with premature MI.** *J. Wessel*<sup>1,2</sup>, *J.J. McCarthy*<sup>2</sup>, *E.J. Topol*<sup>3</sup>. 1) University of California, San Diego, CA; 2) San Diego State University, San Diego, CA; 3) Cleveland Clinic, Cleveland, OH.

Premature MI has been considered to have a stronger genetic basis than late-onset MI. Family history serves as a proxy for the contribution of genetic factors and shared environment. The purpose of the present study is to assess the contribution of family history, clinical covariates and polymorphisms in 19 candidate genes and their association with premature MI. A case series of 469 men and women with MI were dichotomized by age of onset to define premature disease (<55 years men, <65 years women). Different definitions of family history were used to determine which is the strongest predictor of premature MI. One polymorphism each from 19 candidate genes was selected based on their frequency, functionality and missing data. Each polymorphism and clinical covariate was evaluated for their association with premature MI and age of onset of MI. In our series of 223 premature MI cases and 188 late-onset cases, we found parental history of premature MI to be the strongest predictor of premature MI of all of the definitions considered, even after adjustment for other risk factors (odds ratio 1.42,  $p=0.006$ ). High cholesterol ( $p=0.011$ ) and gender ( $p<.0001$ ) were also significantly associated with premature MI. The four most significant polymorphisms ( $p$ 's<.25) (Q534R F5, R478K FGB, 511(G/A) ITGB3 and S290N SELP) were entered into a multivariate model and their contribution reassessed. Only the ITGB3 polymorphism was significantly associated with premature MI (odds ratio 0.43,  $p=0.042$ ), after adjustment for parental history, gender, high cholesterol, FGB and SELP polymorphisms. The SELP polymorphism approached significance ( $p=0.081$ ). When considering their association with age of onset of MI as the outcome, the SELP N allele was associated with a later age of onset (59.92.6 vs. 53.10.9,  $p=0.009$ ). This suggests the ITGB3 and the SELP polymorphisms may be associated with later onset of MI. Family history is still an independent risk factor for premature MI even when accounting for some of the genetic variation that exists.

**Testing for Population Stratification in Case-Control Studies of Asthma Among Latinos.** *N. Coyle<sup>1</sup>, D. Lind<sup>1</sup>, H. Tang<sup>2</sup>, K. Salari<sup>1</sup>, S. Choudry<sup>1</sup>, S. Clark<sup>1</sup>, N. Ung<sup>1</sup>, H. Matellena<sup>1</sup>, P. Avila<sup>1</sup>, J. Casal<sup>3</sup>, A. Torres<sup>3</sup>, S. Nazario<sup>3</sup>, M. Toscano<sup>1</sup>, W. Rodriguez-Cintron<sup>3</sup>, P. Kwok<sup>1</sup>, D. Sheppard<sup>1</sup>, M. Shriver<sup>4</sup>, N. Risch<sup>5,6</sup>, E. Ziv<sup>1</sup>, E. Burchard<sup>1</sup>.* 1) Univ California, San Francisco, San Francisco, CA; 2) Fred Hutchinson Cancer Center, Seattle WA; 3) University of Puerto Rico, San Juan PR; 4) Penn State Univ, Alleghany PA; 5) Stanford Univ, Palo Alto CA; 6) Kaiser Division of Research, Oakland CA.

**Purpose:** Case-control studies are a powerful approach to detect common low to moderate susceptibility alleles for complex diseases. However, such studies may be confounded in populations with substructure and/or recent admixture such as Latinos. We tested whether population stratification may affect the results of case-control studies in Puerto Ricans and Mexican Americans. **Methods:** We compared 93 Puerto Rican asthmatics with 93 healthy Puerto Ricans and 93 Mexican American asthmatics with 93 healthy Mexican Americans. Cases and controls were recruited from the same clinics. We typed 44 markers selected for their high allele frequency differences between West Africans, Europeans and Native Americans. For each population we tested whether the degree of association between pairs of markers on different chromosomes was greater than expected using a permutation test. Population stratification was assessed by comparing the global differences in allele frequencies between cases and controls. **Results:** Among Puerto Ricans, the association between markers on different chromosomes was significantly greater than expected ( $p < 0.0001$ ). Among Mexicans, there was a non-significant trend towards association between markers on different chromosomes ( $p = 0.07$ ). Among Puerto Ricans, 6 of 44 markers were significantly associated with asthma, and there was a significant difference between cases and controls ( $p = 0.019$ ). Among Mexican Americans 2 of 44 markers were associated with asthma and there was no significant difference between cases and controls ( $p = 0.64$ ). **Conclusion:** Asthma case-control studies may be confounded in some admixed populations. Empirical assessment of the effects of stratification will be important to appropriately interpret the results of case-control studies.

**Genetic structure inferred from surnames for all rural localities in Sao Miguel Island (Azores).** *S. Costa, C.C. Branco, R. Cabral, T. Cymbron, P.R. Pacheco, L. Mota-Vieira.* Genetics & Molec Pathol Unit, Hosp Divino Espirito Santo, Azores Islands, Portugal.

Sao Miguel is the biggest (747 Km<sup>2</sup>) and most populated (131,609 inhabitants) island of the Azores, a Portuguese archipelago located in the north Atlantic Ocean. The island includes 2 urban, 8 semi-urban and 44 rural localities (where 50% of the population lives). The mean number of inhabitants and families by rural locality are 1,510 (309-7,407) and 410 (101-1,711), respectively. In the present study, we infer the genetic structure of rural population, using the 2001 telephone directory. In a total of 12,625 subscribers we found 812 different surnames, of which only 92 subscribers have foreign surnames. The highest value of surname diversity was found in Capelas (socio-economically more developed) and Rabo-de-Peixe (the most populated); and the lowest value was found in Lomba de S. Pedro (the least populated). In order to characterize surname diversity, we estimated the coefficient of relationship between localities ( $R_{ij}$ ), the random component of inbreeding ( $F_{st}$ ) for each locality and Nei's genetic distance. The analysis of rural population relationships reveals that Povoacao and Nordeste are the two localities with highest value of  $R_{ij}$  (0.217), whereas Capelas and Nordeste have the lowest value (0.0042). The rural locality displaying the greatest homogeneity is Salga ( $F_{st}=0.0145$ ). The genetic similarity between localities was obtained by a dendrogram based on Nei's distance matrix. We identified two main clusters, one includes Nordeste and Achada, both located in the most eastern part of S. Miguel, and the other includes the remaining 42 localities grouped in three major subclusters. The subcluster that stands out groups all the localities of the western part of the island. These data are consistent with the spatial distribution of surnames obtained by Principal Component (PC) analysis. In fact, the first two PC account for 59% of the total variance and shows a cluster at the upper left corner of the plot with 8 western localities. These findings are valuable for an ongoing project based on DNA sampling of S. Miguel's population. Funded by DRCT-Azores. (lmotavieira@hdes.pt).

**Spatial analysis of the Azorean population based on surname diversity.** *R. Cabral<sup>1</sup>, C.C. Branco<sup>1</sup>, S. Costa<sup>1</sup>, G.U. Caravello<sup>2</sup>, M. Tasso<sup>2</sup>, L. Mota-Vieira<sup>1</sup>.* 1) Genetics & Molec Pathol Unit, Hosp Divino Espirito Santo, Azores Islands, Portugal; 2) Dipartimento di Medicina Ambientale e Sanità Pubblica - sede di Igiene, Università di Padova, Padova, Italy.

Azores is a Portuguese archipelago located in the middle of the Atlantic Ocean. The islands are dispersed over 3 geographic groups: oriental (Sao Miguel and Santa Maria), central (Terceira, Faial, Pico, Graciosa and Sao Jorge) and occidental (Flores and Corvo). Here, we outline the structure of the Azorean population based on surname specificity and distribution. Using the telephone directory (2001), we identified 2,455 different surnames in a total of 55,530 subscribers. In order to characterize the influence of geographic discontinuity on surname diversity, we performed a surname specificity analysis. Our results reveal that each island has surnames 50% more frequent than in the rest of the archipelago (specific surnames). This is particularly evident in the islands of S. Miguel (14 specific surnames with frequency 75%), Santa Maria (2 specific surnames with frequency 80%) and Terceira (2 specific surnames with frequency 90%). Corvo is the only island where a single surname has a value of 100%. To evaluate the genetic similarity between the islands's populations, we then analyzed surname distribution by spatial autocorrelation (Moran's *I*). In a total of 161 surnames, we identified 24 (15%) surnames with statistically significant patterns for 4 distance classes. These patterns were classified as: isolation by distance and depression (37.5%), cline (12.5%), intrusion (12.5%) and long-distance differentiation (8.3%). The remaining 29.2% had no defined pattern. The overall spatial correlogram plot of the 24 surname frequencies shows that the highest Moran's *I* coefficient (0.69) was present in the first distance class (105 Km). Moreover, autocorrelation change from positive to negative for distances greater than 105-250 Km, indicating that mobility is higher between islands of the same geographic group. In population genetic terms, the data show that geographic distance shapes surname diversity among Azores Islands. Funded by DRCT, Azores. (lmotavieira@hdes.pt).

**False positive rates in association studies as a function of marker allele frequency difference between subgroups.**

*D.L. Koller<sup>1</sup>, M. Peacock<sup>2</sup>, T. Foroud<sup>1</sup>, M.J. Econs<sup>1,2</sup>.* 1) Dept of Medical & Molecular Genetics, Indiana Univ Sch Medicine, Indianapolis, IN; 2) Dept of Medicine, Indiana Univ Sch Medicine, Indianapolis, IN.

Uncorrected, racial stratification can result in spurious association findings. This is particularly important for phenotypes with known racial differences such as bone mineral density (BMD), where African American women have significantly higher values as compared with Caucasian women of the same age. We have utilized femoral neck BMD data from a sample of Caucasian (n=381) and African-American (n=126) premenopausal women to examine the effect on association results when the proportion of African American admixture was increased (0%, 1%, 2%, 5%, 10%, 15%, and 20% African-American subjects in the total sample). A genome screen with 373 markers was completed in this sample. Each microsatellite was reduced to a bi-allelic marker, using the most common allele as one allele and binning all other alleles as the second. An ANOVA test of association of BMD was performed in each admixed sample for each marker. We examined the degree to which inflation in the false positive rate (FPR) due to admixture was affected by ethnic differences in the marker allele frequencies, as measured by delta (allele frequency difference/2). We computed the FPR when limiting the biallelic marker set to only those markers with a delta value above the median, and then performed the same analysis for markers with delta values in the lower half of the distribution. For the latter marker set, the inflation was minimal, with FPRs ranging from 0.055 to 0.064 as admixture increased from 1% to 20%. However, for markers in the upper half of the delta distribution, the effect on FPR was quite severe, with a value of 0.122 (compared to the nominal  $\alpha=0.05$ ) for the sample with 20% admixture. The mean delta of the reduced markers in the upper half of the delta distribution is much less (0.12) than the mean delta of all 373 microsatellites before reduction (0.23). This suggests that when multiallelic markers are employed for association tests, the effect of admixture on the FPR would be more severe than the results presented here.

**Determination of Population Structure in the Alzheimers Disease Patient Registry.** *S. Melquist<sup>1</sup>, R. Crook<sup>1</sup>, M. Baker<sup>1</sup>, J. Adamson<sup>1</sup>, R. Mueller<sup>2</sup>, R. Petersen<sup>2</sup>, M. Hutton<sup>1</sup>.* 1) Department of Neurogenetics, Mayo Clinic, Jacksonville, FL; 2) Alzheimer Disease Research Center, Mayo Clinic, Rochester, MN.

The Alzheimers Disease Patient Registry (ADPR), consisting of 300 case and 600 control subjects residing in Olmstead County, Minnesota is potentially a rich resource in genome-wide linkage disequilibrium (LD) mapping of genes involved in Alzheimers Disease (AD). Prior reports of gene variants associated with Alzheimers disease have not been replicated in subsequent studies using other populations (reviewed in 1,2). One explanation for this lack of replication may be that underlying population structure difference between case and control subjects is a confounding factor in the original studies. We hypothesized that population structure may exist in the ADPR samples and underlying structure would be a confounding factor in future AD linkage-disequilibrium studies. Therefore, we examined the extent of population structure in the ADPR series by genotyping a number of unlinked single nucleotide polymorphism and short tandem repeat markers and analyzing the genotype data using the structure program developed by Pritchard and colleagues (3). The results of the population structure analysis of the ADPR series are crucial to future linkage disequilibrium, gene association and epidemiologic studies using this case-control population.

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**Time depth of population subdivision within Africa inferred from linked autosomal marker systems (SNPSTRs).**

*M.J. Jobin, U. Ramakrishnan, J.L. Mountain.* Anthropological Sciences, Stanford University, Stanford, CA.

The population history of Africa prior to the movement of anatomically and behaviorally modern humans out of Africa 50,000-75,000 years ago has had a major impact on the genetic relationships of living populations. Different classes of genetic markers are inconsistent regarding levels of population genetic substructure within Africa. The highly polymorphic mitochondrial and Y chromosomal regions have indicated extensive substructure, while microsatellites and SNPs have not. SNPSTRs are tightly-linked autosomal or X-chromosomal marker systems consisting of one Short Tandem Repeat (STR) polymorphism and at least one Single Nucleotide Polymorphism (SNP). The markedly different mutation rates of the two types of markers decrease homoplasy and increase power to distinguish between hypotheses. These systems therefore provide more detailed information regarding population history than either SNPs or STRs alone. We typed two SNPSTR systems in a set of over 1000 individuals from Africa and other regions of the world. Statistics including  $F_{ST}$  and  $D_{SW}$  along with linkage disequilibrium indicate that subdivision within Africa arose prior to the movement of anatomically and behaviorally modern humans out of Africa 50,000-75,000 years ago. These data are most consistent with mtDNA and Y-chromosomal data and contribute to a growing body of evidence for subdivision within Africa prior to that early migration.

**Use of isolated inbred human populations in genetic epidemiology of complex traits: the Carlantino project.** C. Specchia<sup>1</sup>, P. D'Adamo<sup>2</sup>, M. Carella<sup>5</sup>, L. Zelante<sup>5</sup>, F. Bertoldo<sup>4</sup>, A. D'Eustacchio<sup>2</sup>, A. Ferrara<sup>3</sup>, A. Molinari<sup>3</sup>, P. Gasparini<sup>2</sup>. 1) University of Brescia, Italy; 2) TIGEM, Napoli, Italy; 3) SUN, University of Napoli, Italy; 4) University of Verona, Italy; 5) IRCCS, CSS, San Giovanni Rotondo, Italy.

The use of isolated inbred populations to reduce disease heterogeneity of complex disorders is a valid instrument to study genetic basis of complex diseases. The main objective of this project is the epidemiological description and the genetic analysis of a geographic isolate located in Southern Italy in order to construct linkage disequilibrium maps. Carlantino, a small village settled 5 centuries ago by few founders, was identified. The endogamy rate during last century has been calculated to be 99.5% and three different surnames account for the majority of the living people. In addition birth registers are available from 17th century. A screening of the whole population was performed by a full clinical examination of all individuals including anamnesis, blood pressure measurements, electrocardiogram and bone mineral density evaluation, a clinical chemistry evaluation (blood count plus 20 different biochemical parameters), a development of DNAs and sera banks. Information collection was characterized by a high acceptance rate (94%) from the entire population. Data refer to 1417 Carlantino inhabitants, 615 males and 802 females. Haplotypes study of chromosome Y and of mitochondrial DNA confirmed the presence of 6 couples of founders. Epidemiological data suggest an increased frequency of osteoporosis, hypercholesterolemia, hypertension, heart stroke and diabetes compared to the same region standardized rates. Few preliminary polymorphisms of candidate genes (TGFB, Leptin, AIB1) for bone mass, bone turnover and obesity were studied in the whole sample without any result of genetic association to bone mass. Some families with low bone mass as well as one large pedigree characterized from extremely high bone mass values were identified and will be further investigated. Pedigrees showing the presence of many risk factors for coronary artery disease with and without clinical coronary events will also be studied.

**Influence of past migratory events on Croatian female and male gene pool.** *M. Pericic<sup>1</sup>, L. Barac<sup>1</sup>, H.V. Tolk<sup>2</sup>, I. Martinovic Klaric<sup>1</sup>, S. Rootsi<sup>2</sup>, B. Janicijevic<sup>1</sup>, T. Kivisild<sup>2</sup>, J. Parik<sup>2</sup>, R. Villems<sup>2</sup>, P. Rudan<sup>1</sup>.* 1) Institute for Anthropological Research, Zagreb, Croatia; 2) Department of Evolutionary Biology, Institute for Molecular and Cell Biology, University of Tartu, Tartu, Estonia.

Numerous studies of mitochondrial and Y chromosomal biallelic markers have resolved major prehistoric routes of the settling of Europe and proved that the majority of European gene pool has Paleolithic genes. In order to determine the extent of Paleolithic vs. Neolithic impact among Croatian maternal and paternal lineages, we analyzed biallelic markers diagnostic for main Eurasian haplogroups in 457 Croatian Y chromosomes and 721 mitochondrial DNA of inhabitants from various Croatian regions (mainland and four eastern Adriatic islands: north-Krk; south-Brac, Hvar and Korcula). If Y chromosomal haplogroups: J-12f2, G-201, E-SRY4064 and mitochondrial J and T1 are considered as major Neolithic markers, the majority of females and males in the investigated population are carrying Paleolithic haplogroups. Overall, frequency of mitochondrial Neolithic markers is 11.5%; whereas Y chromosomal Neolithic impact is 13.1%. When investigated populations are analyzed separately, the highest mitochondrial Neolithic impact is noticed in mainland population whereas the lowest is on the island of Korcula. For Y chromosomal markers, the lowest Neolithic impact is noticed in mainland population and the highest on the islands of Krk and Korcula. We will discuss the differentiation between mainland and insular populations in the context of random genetic drift as a predominant stochastic process at the islands. Finally, the obtained results will be reviewed with respect to archeological data relevant to material evidence of Paleolithic and Neolithic legacy in the investigated area, both mainland and islands.

**Study of the stratification of Japanese founding lineages.** *K. Tounai<sup>1</sup>, S. Mano<sup>1</sup>, T. Katoh<sup>1</sup>, B. Munkhbat<sup>1, 2</sup>, H. Ando<sup>1</sup>, G. Oyungere<sup>2</sup>, H. Han<sup>3</sup>, N. Saitou<sup>4</sup>, K. Tokunaga<sup>5</sup>, N. Munkhtuvshin<sup>2</sup>, H. Inoko<sup>1</sup>, G. Tamiya<sup>1</sup>.* 1) Dept. of Molecular Life Science, School of Medicine, Tokai University, Isehara, Japan; 2) Central Scientific Research Laboratory, National Institute of Medicine, Mongolia; 3) Dept. of Microbiology, College of Medicine, The Catholic University of Korea, Seoul; 4) Div. of Population Genetics, National Institute of Genetics, Mishima, Japan; 5) Dept. of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan.

Population stratification may cause a spurious association in a case-control study for complex traits. Generally, the Japanese population is considered to be homogeneous. However, for its origins, a dual structure model is more reasonable on the basis of archaeological and morphological findings. Interestingly, the results of Hammer and Horai (1995) suggest that the presence/absence of a specific Alu insertion on Y-chromosome (YAP) corresponds to descendants of two ancestral populations. Here, we have reexamined the dual structure model using 16 biallelic markers on Y-chromosome and then, using 26 Y-STR markers, assess the levels of differentiation between the two major lineages observed in Japan. Subsequently, we have employed 24 unlinked markers (selected from each autosome) to test for stratification between the Japanese subgroups classified by Y-chromosome haplogroups. We confirmed the existence of two major Y-haplogroups (defined by YAP+ and M175, respectively) in the Japanese population, and found a distinctive differentiation between them for 26 Y-STR markers. On the other hand, the Japanese subgroups classified by Y-chromosomal haplogroups showed no significant differentiation or stratification in the test for 24 unlinked STR loci. Furthermore, we performed a simulation study on the basis of genetic distance,  $R_{st}$ , to verify the decay of stratification suggested by the above results. In conclusion, the dual structure model was supported by our analysis of Japanese Y-chromosomes, but no stratification between two Japanese founding lineages was observed broadly over unlinked loci.

**Mexican American ancestry-informative markers: Examination of population structure and marker characteristics in European Americans, Mexican Americans, Amerindians and Asians.** *M.F. Seldin<sup>1</sup>, H.E. Collins-Schramm<sup>1</sup>, B. Chima<sup>1</sup>, T. Morii<sup>1</sup>, K. Wah<sup>1</sup>, Y. Figueroa<sup>1</sup>, L.A. Criswell<sup>2</sup>, R.L. Hanson<sup>3</sup>, W.C. Knowler<sup>3</sup>, G. Silva<sup>4</sup>, J.W. Belmont<sup>5</sup>*. 1) Rowe Program, Univ California, Davis, CA; 2) Ctr for Arthritis, Univ California, San Francisco, CA; 3) National Institutes of Health, Phoenix, AZ; 4) Obras Sociales del Hermano Pedro, Antigua, Guatemala; 5) Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Markers with large differences in allele frequencies between ethnicities provide ancestry information that can be applied to genetic studies. These markers also provide an opportunity to examine population structure and relationships between populations. We identified over 100 biallelic Ancestry Informative Markers (AIMs) with large allele frequency differences between European Americans (EA) and Pima Amerindians from laboratory and database screens. For 35 of these markers, Mayan, Yavapai and Quechuan Amerindians were genotyped and compared with EA and Pima allele frequencies. Markers with large allele frequency differences between EA and one Amerindian tribe showed only small differences between the Amerindian tribes (e.g. the mean standard variance ( $f$ ) for EA/Pima = 0.36, while Pima/Mayan = 0.02). Examination of structure in individuals demonstrated a clear separation of subjects of European from those of Amerindian ancestry, and a remarkable similarity between individuals from disparate Amerindian populations. The AIMs demonstrated the variation in ancestral composition of individual Mexican Americans, providing evidence of applicability in admixture mapping and in controlling for structure in association tests. In addition, a high percentage of SNPs selected on the basis of large frequency differences between EA and Asian populations had large allele frequency differences between EA and Amerindians (e.g. 13/30 markers with EA/Japanese  $f > 0.3$  had EA/Pima  $f > 0.30$ ), suggesting an efficient method for greatly expanding AIMs for use in admixture mapping/structure analysis in Mexican Americans. Together, these data provide additional support for the practical application of admixture mapping in the Mexican American population.

**Caste, geography and genetic structure in Hindu populations.** *D.J. Witherspoon<sup>1</sup>, T.N. Srinivasan<sup>3</sup>, R. Thara<sup>3</sup>, D.J. Nancarrow<sup>4</sup>, W.S. Watkins<sup>1</sup>, M. Bamshad<sup>1</sup>, B.J. Mowry<sup>2</sup>, L.B. Jorde<sup>1</sup>.* 1) Dept. of Human Genetics, University of Utah, Salt Lake City, UT; 2) Dept. Psychiatry, University of Queensland, Brisbane, Australia; 3) Schizophrenia Research Foundation India, Chennai, Tamil Nadu, India; 4) Queensland Institute of Medical Research, Herston, Brisbane, Australia.

The effect of Hindu caste rank on genetic structure is controversial. Small genetic data sets lack statistical power, while geographical and caste effects can be confounded. In the largest data set yet collected, from upper, middle and lower caste populations in Andhra Pradesh, we found the largest genetic distance between upper and lower castes. In an attempt to replicate these findings in another location, we obtained mtDNA HVS1 sequence from 186 individuals from Tamil Nadu, 800 km to the south. Four groups were sampled in nearly equal numbers: Tamil- and non-Tamil-speaking Brahmins (upper caste), Mudaliar (middle) and Harijan (lower). In both data sets, the upper caste populations had the least genetic diversity, in accord with their presumed lower effective population sizes. By Nei's genetic distance, the non-Tamil-speaking Brahmin, Mudaliar, and Harijan are each most similar to the upper, middle and lower caste groups from AP, respectively. This suggests that caste divisions outweigh geographical effects at this range. The Tamil-speaking Brahmin are unusual in that they are most similar to the lower caste group from Andhra Pradesh and most different from the upper-caste group. (Support: NSF SBR-9514733, SBR-9512178, SBR-9818215)

**Nei's genetic distance (x100,000) between caste groups in Andhra Pradesh (rows) and Tamil Nadu**

	NonTamil	Brahmin	Mudaliar	Harijan	Tamil Brahmin
Upper	27	55	63	71	
Middle	37	44	45	60	
Lower	45	72	33	54	

**The gender effect in juvenile Huntington disease patients of Italian origin.** *M. Cannella<sup>1</sup>, C. Gellera<sup>2</sup>, V. Maglione<sup>1</sup>, P. Giallonardo<sup>1</sup>, G. Cislighi<sup>3</sup>, M. Muglia<sup>4</sup>, A. Quattrone<sup>4</sup>, F. Pierelli<sup>5</sup>, S. Di Donato<sup>2</sup>, F. Squitieri<sup>1</sup>.* 1) Neurogenetics Unit, IRCCS NEUROMED, POZZILLI, IS, Italy; 2) . Division of Biochemistry and Genetics, Neurological Institute IRCCS C. Besta, Milan, Italy; 3) Division of Neurology, Fornaroli Hospital, Magenta (MI), Italy; 4) . Institute of Experimental Medicine and Biotechnology, National Research Council (CNR), Piano Lago Mangone (CS), Italy; 5) Dept. of Neurological Sciences, University La Sapienza, Rome.

We analyzed a population of juvenile Huntington disease (HD) subjects of Italian origin (n = 57). The main aim of this study was to analyze the gender effect of the affected parent on age at onset and clinical presentation of offspring with juvenile HD. We also analyzed molecular features of the disease, including CAG mutation length and GluR6 gene polymorphism, according to the affected parents gender. The mutation length was longer in paternally than in maternally transmitted HD juvenile patients (p = 0.025), nevertheless a similar mean early onset in the two groups (p > 0.05). This data was even enforced by that obtained from the whole cohort of patients included in the databank (n = 600) where, in the presence of increased mean parent-child CAG repeat change in paternal vs maternal meiotic transmissions (+ 7.3 vs + 0.7 CAG, p = 0.0002), the mean parent-child year-of-onset change was similar in the two groups (-10.4 and 7.0 years, p > 0.05). A lower TAA-triplet in GluR6 was associated with an earlier age at onset in juvenile patients (p = 0.031, R<sup>2</sup> = 0.10). When we added the GluR6 effect on age at onset to the CAG expanded number effect (p = 0.0001, R<sup>2</sup> = 0.68) by multiple regression approach, the coefficient of determination R<sup>2</sup> increased to 0.81. This effect in addition to the expanded CAG repeat number, found in juvenile and not in adult patients, was slightly enforced by paternal compared to maternal transmissions (R<sup>2</sup> 0.82). Our findings suggest the occurrence of a weaker effect of the paternal mutation on juvenile age at onset in our population, possibly amplified by other genetic factors, such as the TAA-triplet length in the GluR6 gene.

**Segregation Analysis of Intraocular Pressure in the Beaver Dam Eye Study.** *P. Duggal<sup>1</sup>, A.P. Klein<sup>1</sup>, K.E. Lee<sup>2</sup>, R. Klein<sup>2</sup>, J.E. Bailey-Wilson<sup>1</sup>, B.K. Klein<sup>2</sup>.* 1) Statistical Genetics Section, IDRB/NHGRI/NIH, Baltimore, MD; 2) Dept of Ophthalmology and Visual Sciences, University of Wisconsin-Madison Medical School.

Primary open-angle glaucoma is a leading cause of blindness in the world. In the US, it is estimated that 3 to 6 million people have elevated intraocular pressure (IOP) and are at an increased risk for developing glaucoma. To investigate a potential genetic contribution to IOP, we performed a complex segregation analysis on 2,337 individuals in 620 extended pedigrees ascertained through a population-based cohort, the Beaver Dam Eye Study in Beaver Dam, Wisconsin. Detailed medical histories and eye examinations were performed on all participants. Intraocular pressure was measured with a Goldmann applanation tonometer in each eye. The higher IOP measurement between the eyes was used as a continuous trait, and treatment with drops and age were covariates. Analyses were conducted using regressive class D models in REGC in S.A.G.E 3.1. This segregation analysis does not support a single major Mendelian locus for IOP since these models were rejected. The most parsimonious model was an environmental mixed model with the transmission probabilities ( $\tau$ ) equal to the allele frequency. This model was not rejected ( $p=0.39$ ) when compared to a totally unrestricted (general) model. Another version of the general model (semi-general) commonly used to test for the presence of a major locus effect in a mixed model is one in which the  $\tau$  of the A allele for AA and BB genotypes are fixed to 1.0 and 0, and the remaining parameters are unrestricted. This model was not rejected ( $p=0.13$ ) and yielded an estimate of the  $\tau_{AB}$  genotype of 0.66, close to its Mendelian value of 0.5. Since we could not reject the environmental mixed model with equal  $\tau$ s it is plausible that there are non-transmitted or environmental factors that influence IOP. When the Mendelian dominant mixed model was compared to the semi-general model, the dominant model was marginally non-significant ( $p=0.056$ ). This is consistent with a multifactorial model that may include multiple genes and environmental factors that contribute to IOP.

**Molecular testing of buccal cell DNA is the method of choice for Tay Sachs Disease screening in Ashkenazi Jewish high schools.** *A. Bankier*<sup>1, 2, 3</sup>, *A. Gason*<sup>1</sup>, *E. Sheffield*<sup>2</sup>, *V. Petrou*<sup>2</sup>, *M.B. Delatycki*<sup>1, 2, 3</sup>. 1) Genetic Health Services Victoria, RCH, Victoria, Australia; 2) Bruce Lefroy Centre for Genetic Health Research, RCH, Victoria, Australia; 3) Murdoch Childrens Research Institute, RCH, Victoria, Australia.

Tay Sachs disease is an autosomal recessive neurodegenerative condition that is more common in the Ashkenazi Jewish community and generally results in those affected dying before five years. Carrier screening programs have been very successful in reducing the incidence of this disease. Carrier screening has traditionally been done by measuring levels of hexosaminidase A in blood. Molecular testing for three common mutations allows over 98% of Ashkenazi Jewish carriers to be identified. Some have argued that enzyme analysis should nevertheless be used, as ancestry is sometimes uncertain. We have conducted a screening program in Jewish day schools since 1997. Screening has been by enzyme analysis until this year where we have changed to buccal swab molecular DNA testing. From 1998 to 2003 the education and testing process has been identical apart from the testing method in 2003. Students receive education and 2-10 days later are offered screening after one to one counselling. Testing is free of charge. We surveyed students who chose not to be tested in 2002 as to whether they would have done so had testing been by buccal swab rather than venesection. 16/24 (66.7%) indicated that they would have done so. From 1999-2002, 780/965 (81%) of students had testing by blood enzyme analysis. In 2003, 222/229 (97%) of students had testing by buccal swab and molecular analysis. This is a significant increase in uptake ( $\chi^2=35.6$ ;  $p<10^{-4}$ ). Therefore, more students who wish to have testing do so where it is offered by buccal swab rather than venesection.

**Adult genetic services generate revenue for a university medical center.** *R.L. Bennett<sup>1</sup>, D.L. Olson<sup>1</sup>, P.H. Byers<sup>1,2</sup>.*  
1) Division of Medical Genetics, University of Washington, Seattle, WA; 2) Department of Pathology.

Services provided to 834 patients seen at the Adult Medical Genetics Clinic (AMGC) at the University of Washington Medical Center (UWMC) were tracked from July 2001 through June 2002. Patients were seen with 150 different diagnoses. There were 1093 visits, a 376% increase from 1996. Fifty-seven percent of patients were new to the UWMC at the time of referral to the AMGC. Thirty-six percent of patients had procedures or laboratory studies ordered at their AMGC visit--44% of these laboratory studies were performed at UWMC, and 90% of the procedures ordered were performed at UWMC. AMGC providers made 80 patient referrals to other UWMC clinics. The vast majority of patients seen during the study period were referred by an outside provider; only 18% of patients were referred to AMGC by a UWMC provider. In addition to the fees generated for AMGC visits, the revenue generated for laboratory tests performed at UWMC was \$35,000-\$36,000, \$77,000-94,000 for procedures done at UWMC, and \$38,000-43,000 for new consultations with other UWMC providers. These data indicate that an adult genetics clinic is an entry to medical care for patients with complicated medical diseases, many of whom will continue their care with other health care providers at the medical center. The services coordinated through a medical genetics clinic generate substantial revenue and bring prestige to the medical center.

**Recurrent *de novo* mitochondrial DNA mutations in respiratory chain deficiency.** *S. Lebon<sup>1</sup>, M. Chol<sup>1</sup>, P. Bénit<sup>1</sup>, C. Mugnier<sup>1</sup>, D. Chretien<sup>1</sup>, I. Giurgea<sup>1</sup>, I. Kern<sup>3</sup>, E. Girardin<sup>3</sup>, L. Hertz-Pannier<sup>2</sup>, P. de Lonlay<sup>1</sup>, A. Rötig<sup>1</sup>, P. Rustin<sup>1</sup>, A. Munnich<sup>1</sup>.* 1) INSERM U393 and Department of Genetics, Hôpital Necker, Paris, France; 2) Department of Pediatric Radiology, Hôpital Necker, Paris, France; 3) Department of Pediatrics, Hôpitaux Universitaires de Genève, Genève, Switzerland.

Starting from a cohort of 50 NADH-oxidoreductase (complex I) deficient patients, we carried out the systematic sequence analysis of all mitochondrially-encoded complex I subunits (ND1 to ND6 and ND4L) in affected tissues. This approach yielded the unexpectedly high rate of 20% of mutation identification in our series. Heteroplasmic recurrent mutations included two hitherto unreported (T10158C and T14487C) and three previously reported mutations (T10191C, T12706C and A13514G) in children with Leigh or Leigh-like encephalopathy. Surprisingly, the recurrent mutations consistently involved T-C transitions (p10-4). Why specific mitochondrial (mt) DNA residues (thymine and guanine) were recurrently targeted in respiratory chain deficiency remains unexplained. One can hypothesize that the thymine and guanine residues were more vulnerable to ROS-induced oxidative damages into the mitochondria. Whatever the mechanism, this study supports the view that an efficient molecular screening should be based on an accurate identification of respiratory chain enzyme deficiency.

**Folate Metabolizing gene polymorphisms and Head and Neck cancer.** *H. Chen<sup>1</sup>, K. Yanamandra<sup>1</sup>, J. Rodriguez-Paris<sup>2</sup>, M. Smith<sup>2</sup>, D. Napper<sup>1</sup>, T.F. Thurmon<sup>1</sup>, S.A. Ursin<sup>1</sup>, R. Dhanireddy<sup>1</sup>, J.A. Bocchini Jr.<sup>1</sup>, G.M. Mills<sup>2</sup>, J. Glass<sup>2</sup>.*  
1) Dept Pediatrics, Perinatal Gen, LSUHSC- Shreveport, Shreveport, LA; 2) Feist-Weiller Cancer Center, LSH Health Sciences Center, Shreveport, LA.

We have a long-standing interest in the relationship of Methylene tetrahydrofolate reductase (MTHFR) genotypes and birth defects. We found a significant association of maternal MTHFR genotypes with Trisomy 18 but not with Down syndrome pregnancies. Then, we extended our studies to the associations of folate and cobalamin metabolizing gene polymorphisms with orofacial clefts, vascular conditions such as retinal vascular occlusions, and recently, to cancer etiology. We found significant associations between maternal MTHFR genotypes and cleft palate, and between patient MTHFR genotype and retinal vascular occlusions. In studies in West Africa, we found a much lower frequency of MTHFR mutant genotypes and the correlation with orofacial clefts was less significant. This implied an ethnocentric mechanism for the association. In the present research project we studied over 300 non-Hispanic white patients with various forms of cancer including over 80 patients with Head and Neck cancer to investigate the relationship of MTHFR genotypes and the cancer etiology, using allele specific multiplex PCR genotyping method. Our pilot data showed 11% 677TT mutant homozygotes, 52% 677CT Heterozygotes, and 36% 677CC normal homozygotes in Head and Neck cancer patients compared to control frequencies of 13%, 45%, and 42%, suggesting similar frequencies of MTHFR mutant genotypes between Head and Neck cancer and controls. This is the first study of this type. If our data are verified by other centers, there is neither association of MTHFR mutant genotype with this cancer (odds ratio 1.1) nor protection by normal genotype. Data on various cancer types, genotypes, and statistics will be presented.

**The Manitoba Experience with *GJB2* & *GJB6* Genetic Testing.** *T.L. Dyck*<sup>1</sup>, *S.L. Marles*<sup>2,3</sup>, *J. Gorin*<sup>2</sup>, *E. Grudeski*<sup>1</sup>, *E.L. Spriggs*<sup>1,2,3</sup>. 1) Molecular Diagnostic Lab, Health Sciences Centre, Winnipeg, Canada; 2) Section of Genetics & Metabolism, Health Sciences Centre, Winnipeg, Canada; 3) Depts. of Pediatrics & Child Health and Biochemistry & Medical Genetics, Univ. of Manitoba, Winnipeg, Canada.

Non-syndromic hearing loss (NSHL) accounts for 70% of hereditary deafness. Mutations in the *GJB2* gene are the predominant cause of the autosomal recessive form of NSHL. The most common mutation, 35delG, accounts for half of all *GJB2* mutations. Ethnic-specific mutations, including 235delC in the Asian population, and 167delT in the Ashkenazi Jewish, have also been identified. Manitoba, a Canadian province, has a unique population with a number of different ethnic groups. The objective of this study was to determine the prevalence of *GJB2* and *GJB6* mutations in Manitobans with autosomal recessive NSHL. Fifteen unrelated patients with bilateral hearing loss and no evidence of syndromic or environmental involvement were accepted into the study. All patients were tested for the 35delG and 167delT mutations. If no mutation or only one was found, the entire gene was sequenced. One third of the patients had two *GJB2* mutations. Four were homozygous for the 35delG mutation. One child of part Japanese ethnicity was determined to be a compound heterozygote with the Asian 235delC mutation and a rare E120 mutation. Another patient was heterozygous for the M34T mutation; the significance of this mutation is unclear as it has been reported in the literature as an autosomal dominant, an autosomal recessive, as well as a non-disease causing change. Given the recent finding regarding the contribution of *GJB6* to NSHL, all patients were also analyzed for the common 342 Kb deletion in the *GJB6* gene. To date, no patients were found to harbour this deletion or any other *GJB6* mutation. From our results it can be concluded that 35delG is the most common mutation in our population. Due to the diversity of ethnic groups in Manitoba, sequence analysis of the *GJB2* gene should be performed on all patients as the compound heterozygote (235delC/E120) would not have been identified if analysis had stopped when no 35delG mutations were detected. Furthermore, *GJB6* does not appear to be a common cause of NSHL in Manitoba.

**Genetic diagnosis in congenital deafness: molecular analysis of Connexin 26, Connexin 30 and Pendrin. R.**

*BERNARD<sup>1</sup>, S. SIGAUDY<sup>1</sup>, S. ROMAN<sup>2</sup>, D. TAULIER<sup>1</sup>, JM. TRIGLIA<sup>2</sup>, N. LEVY<sup>1, 3</sup>.* 1) Departement de Genetique Medicale, Hopital d'enfants de la Timone, Marseille, France; 2) Service d'Oto-Rhino-Laryngologie pediatrique, Hopital d'enfants de la Timone, Marseille, France; 3) Inserm U491 Genetique Medicale et Developpement, Faculte de Medecine de la Timone, Marseille France.

Despite the fact that hereditary hearing loss is a frequent and heterogeneous disorder, high prevalence of connexin 26 (GJB2) mutations in congenital isolated deafness has been demonstrated. More recently the implication of connexin 30 (GJB6), located also on chromosomal region 13q12, has been reported in some deaf families in association with Cx26 mutations, suggesting a possible complexity of the mechanisms responsible for some congenital cases of deafness, such as digenism. In addition, the Pendrin (PDS) gene is known to be responsible for the Pendred syndrome, which is an autosomal recessive form of deafness associated with developmental abnormalities of the cochlea and goiter. However, the PDS gene could be also involved in non syndromic hearing loss, though its frequency in this case remains to be determined. Here we present the study of a serie of 52 unrelated deaf patients, clinically evaluated and explored by molecular analysis of Cx26, Cx30, and PDS. We report an original method for rapid detection of the most frequent mutation of Cx26, the 35delG, based on fluorescent PCR. Our study allowed the identification of Cx26 mutations in 30% of the cases ; deletion of Cx30 was observed in 2 patients, and PDS analysis identified genomic variations in six patients. Our results include genotype-phenotype correlations and tend to raise up again the difficult questions of molecular strategy and genetic counselling in heterogeneous non lethal disorders, such as neurosensory disorders.

**Connexin-26 mutations associated with the most common form of non-syndromic neurosensory autosomal recessive deafness (DFNB1) in the Iranian Population.** *M. Mohseni*<sup>1</sup>, *S. Arzhangi*<sup>1</sup>, *R.A. Cucchi*<sup>2</sup>, *Y. Riazalhosseini*<sup>1</sup>, *M. Malekpour*<sup>1</sup>, *A. Daneshi*<sup>3</sup>, *M. Farhadi*<sup>3</sup>, *K. Kahrizi*<sup>1</sup>, *Y. Shafeghati*<sup>1</sup>, *R.J.H. Smith*<sup>2</sup>, *H. Najmabadi*<sup>1</sup>. 1) Genetics Research Center, The Social Welfare and Rehabilitation Sciences University, Tehran, Iran; 2) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa City, IA, USA; 3) Department of Otolaryngology, Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran.

The incidence of congenital hearing loss is approximately 1 per 1000-2000 infants. In many populations of European descent, mutations in *GJB2* (chromosomal locus 13q11-q12) encoding the gap-junction protein connexin-26 (Cx26) are the most common known cause of severe-to-profound deafness that is inherited in an autosomal recessive fashion (DFNB1). One mutation in *GJB2* (35delG) accounts for approximately two-thirds of all Cx26 mutations in DFNB1 patients. We also established the efficacy of Denaturing High Performance Liquid Chromatography (DHPLC) as a screening assay for detecting mutations in the coding region of *GJB2* in 220 patients with hereditary hearing impairment. Sequence confirmation was 100% concordant with DHPLC and identified allele variants in 21 patients. These mutations included: 167delT, R184P, R32H, - 3170GA, delE120, V153I, V27I, 329delA, I69I, W24X, R127H. V153I (in 9 patients) and V27I are common polymorphisms. 329delA and I69I are novel allele variants. We screened patients heterozygous for only one *GJB2* allele variant (12 probands), as well as 103 patients with wild-type alleles, for (*GJB6-D13S1830*). No deletion was detected in any patient. These data suggest that other loci may make major contributors to the ARNSHL genetic load in the Iranian population.

**Assessing *GJB2/GJB6* testing and counseling as adjunct to newborn hearing screening.** C. Palmer<sup>1</sup>, A. Martinez<sup>1</sup>, M. Fox<sup>1</sup>, B. Crandall<sup>1</sup>, N. Shapiro<sup>1</sup>, M. Telatar<sup>1</sup>, Y. Sininger<sup>1</sup>, W. Grody<sup>1</sup>, L. Schimmenti<sup>2</sup>. 1) UCLA, Los Angeles, CA; 2) U Minn, Minneapolis, MN.

Introducing genetic information into the early hearing detection and intervention (EHDI) process may streamline diagnosis and treatment of infants with hearing loss. Here we describe our early experience in a 4y, prospective, longitudinal study in an ethnically diverse population to assess the timing and impact of *GJB2/GJB6* testing and counseling in the EHDI process. Testing is offered at 2 different points: 1) after failure of newborn screening but before final diagnosis (prediagnosis) and 2) after diagnosis of hearing loss (postdiagnosis). Families are eligible if the child with (potential) hearing loss has no other medical conditions and is <2y. Following informed consent through parental genetic counseling, the child's genomic DNA is subject to mutation analysis (*GJB2*: 35delG, 235delC, 167delT; *GJB6*: 342kb del) and *GJB2* sequencing as needed. Family history and demographic data are collected. Questionnaires assessing parental anxiety, perceived personal control, risk perception, attitudes toward genetic testing, and understanding are completed at enrollment, 1m-and 6m-post genetic counseling of results. Genetic evaluation is performed, medical and audiology records are obtained for up to 4y. To date, 9 ethnically diverse families, with no other history of congenital hearing loss, are enrolled in the postdiagnosis group. At enrollment, all parents (n=12) felt that genetic testing could help them understand why their child has hearing loss, and the majority felt that it was somewhat likely that their child had *GJB2/GJB6*-related hearing loss. 5 babies were found to have *GJB2*-related hearing loss; 1m after disclosure, these parents responded that their child definitely had *GJB2*-related hearing loss. Hearing loss of 4 babies was not explained by genetic testing; 1m after disclosure, these parents responded that it was not at all likely that their child had *GJB2/GJB6*-related hearing loss, and that testing did not help them understand the cause of the hearing loss. This early experience indicates parental interest in, and reasonable understanding of, genetic testing and results.

**(GJB6-D13S1830) is not a frequent DFNB1-causing allele in the Iranian population.** *Y. Riazalhosseini*<sup>1, 2</sup>, *Kh. Jalalvand*<sup>1</sup>, *S. Arzhangi*<sup>1</sup>, *M. Mohseni*<sup>1</sup>, *C. Nishimura*<sup>3</sup>, *N. Mahdieh*<sup>1</sup>, *A. Ebrahimi*<sup>1</sup>, *H. Najmabadi*<sup>1</sup>, *R.J.H. Smith*<sup>3</sup>. 1) Genetics Research Center, The Social Welfare and Rehabilitation Sciences University, Tehran, Iran; 2) Khatam University, Tehran, Iran; 3) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa City, IA, USA.

Mutations in the gene that encodes the gap-junction protein connexin 26 (*GJB2*) at the DFNB1 locus on chromosome 13q12 are the major cause of autosomal recessive non-syndromic sensorineural deafness (ARNSD) in many different populations. A fraction of patients with *GJB2* mutations have only one mutant allele, and in some familial cases with linkage to the DFNB1 locus no mutations in *GJB2* are reported. Recently, a large deletion involving the *GJB6* gene encoding connexin 30 which is also located at the DFNB1 locus ((*GJB6-D13S1830*)) has been reported to cause ARNSD in homozygotes for this mutation and in compound heterozygotes carrying deafness-causing allele variants of *GJB2* on the opposite allele. To evaluate the importance of (*GJB6-D13S1830*) in the Iranian population, we screened 115 deaf probands with ARNSD from various regions of the country. One hundred three of them were deaf probands with normal *GJB2* alleles, the remaining 12 were heterozygote for only one *GJB2* mutation. Screening for (*GJB6-D13S1830*) was completed using PCR primers that amplify the breakpoint junction of this deletion (NEJM 2002; 346:243-9). (*GJB6-D13S1830*) was not detected in our subjects. We conclude that (*GJB6-D13S1830*) is not a frequent DFNB1-causing allele in the Iranian population.

**Mutation screening of the calcium-sensing receptor (CASR) gene by denaturing HPLC (dHPLC) in the diagnosis of inherited disorders of parathyroid hormone metabolism.** *R.A. Booth<sup>1</sup>, B.Y.L. Wong<sup>2</sup>, A. Scillitani<sup>3</sup>, A. Shuen<sup>1</sup>, L. Fu<sup>2</sup>, C. Wei<sup>4</sup>, G.N. Hendy<sup>5</sup>, D.E.C. Cole<sup>1,2</sup>.* 1) Dept. of Laboratory Medicine & Pathobiology, Univ. of Toronto, Toronto, Canada; 2) Dept. of Clinical Pathology, Sunnybrook & Womens College Health Sciences Centre, Toronto, Canada; 3) Dept. of Endocrinology, Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; 4) Toronto Medical Laboratories, University Health Network, Toronto, Canada; 5) Dept. of Medicine, Physiology & Human Genetics, McGill Univ., Montreal, Canada.

Activating mutations of the CASR gene cause autosomal dominant hypoparathyroidism with hypercalciuria. When they arise de novo, the clinical picture is one of idiopathic hypoparathyroidism, but germline mosaicism may alter recurrence risks. Inactivating mutations are associated with hypercalcemia and the phenotype is usually benign, although homozygosity manifests as neonatal severe hyperparathyroidism. Recently, however, mutations have also been identified in some kindreds with familial isolated hyperparathyroidism. To address the question of whether CASR mutations underlie some cases of sporadic primary hyperparathyroidism (PHPT) as well, we developed a high-throughput mutation screening protocol using dHPLC. Genomic DNA from 137 cases of PHPT, in which other genetic causes of hyperparathyroidism (MEN1, HPT with jaw tumors) had been rigorously excluded, were PCR-amplified to produce a series of amplicons covering exons 2 to 7 of CASR, as well as their exon-intron boundaries. Screening exon 4, we identified heterozygosity for a novel T263M mutation that disrupts an N-glycosylation consensus sequence (Asn-X-Ser/Thr) and is predicted (by NetNGlyc1.0) to knock out N-glycosylation at Asn261. Glycosylation of the calcium-sensing receptor appears to be required for a full response to extracellular calcium. Otherwise, known polymorphisms are readily distinguished by our dHPLC protocol, but novel heterozygosity has been observed in exon 4 and exon 7 in two additional PHPT samples, respectively, and mutational analysis is underway. Full screening of all exons will provide a clearer idea of the minimal mutation yield that can be expected of dHPLC screening in this parathyroid disorder.

**Is there a gender difference in the population prevalence of Factor II gene G20210A polymorphism?** *D. Napper, K. Yanamandra, S.A. Ursin, H. Chen, R. Dhanireddy, J.A. Bocchini Jr., T.F. Thurmon.* Dept Pediatrics, Louisiana State Univ Med Ctr, Shreveport, LA.

Venous thrombosis is caused by genetic and non-genetic factors. Several polymorphic gene factors are implicated in thrombophilia including Factor II G20210A, Factor V Leiden, and Methylenetetrahydrofolate reductase (MTHFR) C677T. The Factor II gene is 21 kb in size and is localized on chromosome 11p11-q12. The gene has 14 exons with a 5'-upstream and a 3'-untranslated regions. Poort et al in 1996 from Leiden, the Netherlands, identified a polymorphic marker, G20210A in the 3'-UT region of the gene and found the polymorphism was associated with increased risk for venous thrombosis. The population frequency of G20210A polymorphism varies with ethnicity. Many of the reports showed a very rare to none 20210AA homozygotes in the general population. The Leiden thrombophilia study revealed a 2.3% (11 out of 474) 20210GA heterozygosity among the Dutch Caucasians. According to the Centers of Diseases Control (CDC) Atlanta, Georgia study the heterozygote frequency was 1 in 318 African-Americans (0.3%). A Brazilian study revealed 1.6% (2 in 120) heterozygosity in whites, none in 112 Blacks, none in 148 Amerindians, or 40 Japanese. So far there was no mention of population prevalence figures stratified by gender for the Factor II G20210A polymorphism. In the course of our present investigation of Factor II polymorphism studies in the etiology of cancer we found a nonsignificant but more than doubling (3% vs 1.3%) of the heterozygous 20210GA frequency in our NW Louisiana female healthy infants compared to male infants (Chi Sq 0.5,  $p=0.5$ , odds ratio  $>2.3$ ). If our data are verified by other centers, then any association studies involving Factor II gene polymorphism must not only stratify the genotypes by ethnicity but also by the gender to get a meaningful relationship. Data on genotypes by race and by gender, and statistics will be presented.

**Methylenetetrahydrofolate reductase (MTHFR)C677T polymorphism and Lung cancer.** *T. Thurmon<sup>1</sup>, K. Yanamandra<sup>1</sup>, S.A. Ursin<sup>1</sup>, H. Chen<sup>1</sup>, D. Napper<sup>1</sup>, R. Dhanireddy<sup>1</sup>, J.A. Bocchini Jr.<sup>1</sup>, J. Rodriquez-Paris<sup>2</sup>, M. Smith<sup>2</sup>, G.M. Mills<sup>2</sup>, J. Glass<sup>2</sup>.* 1) Dept Pediatrics-Genetics, Louisiana State Univ, Shreveport, LA; 2) Feist-Weiller Cancer Center, LSU Health Sciences Center, Shreveport, LA.

Folate deficiency has been implicated in the etiology of birth defects, cardiovascular anomalies, stroke, and recently, tumorigenesis of solid tumors such as breast, cervix, colon, pancreas, and liver. There has been very little study of enzyme gene polymorphisms in the shared folate and cobalamin (vitamin B12, another B vitamin) metabolic pathway in the etiology of cancer. Since Folate and Cobalamin metabolizing enzymes share the C1 metabolic pathway, we are studying the methylenetetrahydrofolate reductase (MTHFR) C677T, methionine synthase reductase (MTRR) A66G, and methionine synthase (MS) A2756G polymorphisms. This report concerns MTHFR. Over 200 non-Hispanic white cancer patients have been investigated to study the relationship of MTHFR genotypes with various forms of cancer, using allele specific multiplex PCR genotyping method. Overall, there were 14% 677TT homozygotes, 46% 677CT heterozygotes, and 40% 677CC homozygotes compared to control frequencies of 13%, 45%, and 42%. Previous studies have shown that low folic acid or mutant MTHFR genotypes could be risk factors in lung cancer. However, our data suggests similar frequencies of MTHFR mutant genotypes between lung cancer and controls showing neither association of MTHFR mutant genotype with lung cancer (odds ratio 1.1) nor protection by the normal genotype, in contrast to reports in the literature. Data on various cancers, genotypes, and statistics will be presented.

**Factor II G20210A polymorphism and Breast Cancer.** *S.A. Ursin<sup>1</sup>, K. Yanamandra<sup>1</sup>, J. Rodriquez-Paris<sup>2</sup>, M. Smith<sup>2</sup>, D. Napper<sup>1</sup>, T.F. Thurmon<sup>1</sup>, H. Chen<sup>1</sup>, R. Dhanireddy<sup>1</sup>, J.A. Bocchini Jr.<sup>1</sup>, G.M. Mills<sup>2</sup>, J. Glass<sup>2</sup>.* 1) Pediatrics, Perinatal Gen Sec, LSUHSC, Shreveport, LA; 2) Feist-Weiller Cancer Center, LSU Health Sciences Center, Shreveport, LA.

Venous thrombosis is caused by genetic and non-genetic factors. Several polymorphic gene factors are implicated in thrombophilia including Factor II G20210A, Factor V Leiden, and Methylene tetrahydrofolate reductase (MTHFR) C677T. The Factor II gene is 21 kb in size and is localized on chromosome 11p11-q12. The gene has 14 exons with a 5'-upstream and a 3'-untranslated regions. Poort et al in 1996 from Leiden, the Netherlands, identified a polymorphic marker, G20210A in the 3'-UT region of the gene and found the polymorphism was associated with increased risk for venous thrombosis. The polymorphic frequency of 20210GA genotype has been found to be 1-2% in Caucasians and less than 0.2-0.3% in African-Americans. Although Factor II polymorphism was found to be a risk factor for vascular diseases, its association with cancer etiology was not studied. In the present investigation we studied the association of thrombophilic polymorphic gene markers in the etiology of cancer. We genotyped over 200 cancer patients and over 2300 controls. Previous studies in the literature showed negligible number of 20210AA homozygotes in the population. Similarly, we have not found any mutant 20210AA homozygotes. When we stratified the data by ethnicity and gender we found a significant increase of mutant 20210GA genotypes in Caucasian breast cancer patients over Caucasian controls 9.7% vs 2.1% (Chi Sq 3.7,  $p < 0.05$ , odds ratio 4.9). When combined with our data on lung cancer, the results were 6.4% compared to 1.9% in controls (Chi sq 5.5,  $p < 0.02$ , odds ratio 3.6). Our data showed that 20210GA genotype is a significant risk factor in the etiology of breast cancer. This is the first report in the literature. Genotypes and cancer data among different ethnicities will be presented.

Program Nr: 1362 from 2003 ASHG Annual Meeting

**Five years experience of a diagnostic service for craniofacial syndromes.** *R.L. Jones, E.M. Craig, C.J. McMahon, G.M. Monaghan, M. Boxer, R.M. Winter, C.G. Norbury.* Regional Genetics Service, Great Ormond Street Hospital, London, UK.

The NSCAG-funded craniofacial genetics service was established at Great Ormond Street Hospital in October 1997. Since then we have tested around 900 patient samples for a variety of craniosynostosis syndromes associated with the FGFR and TWIST genes. Mutation screening cascades have been developed for the various disorders, in order to maximise the efficiency of screening for future samples. Mutation detection rates vary widely by condition, for example mutations were found in only 3.5% of referrals requesting only the common FGFR3 P250R mutation, associated with Muenke syndrome (non-syndromic craniosynostosis), compared to 60% of Saethre-Chotzen syndrome cases, over 70% of patients with a definite clinical diagnosis of Crouzon or Pfeiffer syndrome, and 100% of confirmed cases of Apert syndrome. Genotype-phenotype correlations will be discussed. Testing for familial mutations and prenatal diagnosis is offered when a mutation has been identified, and prenatal diagnosis is also offered for abnormal ultrasound findings. So far around 20 cases of prenatal diagnosis have been performed as part of the service. The service continues to expand, and new and future developments (e.g. CE SSCP) will also be discussed.

**Test error tolerance and physician-offspring concordance in attitudes toward Alzheimers Disease (AD) genetic susceptibility testing.** *G. Chase<sup>1</sup>, M.D. Fallin<sup>3</sup>, S.S. Bassett<sup>2</sup>*. 1) Dept of Health Evaluation, Penn St Univ College of Med , Hershey, PA; 2) Dept of Psychiatry, Johns Hopkins School of Medicine, Baltimore, MD; 3) Dept of Epidemiology, Johns Hopkins School of Publi Health.

We studied 107 adult offspring of AD cases ascertained in a genetic linkage study and also administered parallel questionnaires regarding various aspects of genetic susceptibility testing to the physicians who treated their affected parents. This report is concentrated on the question of whether attitudes and opinions about such testing were concordant between offspring and medical care providers as well as with determinants of test error tolerance among adult offspring. Tolerance for tests with high error rate was predicted by male gender, low education and concern with financial planning. Concern with financial planning as a reason for taking an AD susceptibility test was not associated with male gender among offspring of AD patients. The predictive importance of male gender for high test error tolerance is not attributable to greater financial concerns among male offspring; both factors predict this outcome independently. There is a marked discrepancy between the physicians' report on importance of discussing genetic factors in AD with offspring and the offspring reporting that such discussion actually took place. Although there was no greater than a chance association between offspring and physicians on the issue of preference for the latest tests and treatments, there was substantial agreement between offspring and physicians on reasons for taking an AD susceptibility test.

**Alpha synuclein gene dosage in familial and sporadic Parkinson's disease.** *J.O.M. Johnson<sup>1</sup>, M.J. Hanson<sup>3</sup>, S. Hague<sup>1</sup>, A. Singleton<sup>2</sup>, A. Crawley<sup>2</sup>, B. Ravina<sup>2</sup>, J. Hardy<sup>3</sup>, K. Gwinn-Hardy<sup>2</sup>, A. Singleton<sup>1</sup>.* 1) National Institute on Aging, National Institutes of Health, Bethesda, MD; 2) Neurogenetics Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD; 3) Laboratory of Neurogenetics, National Institutes of Health, Bethesda, MD.

The first reported linkage for Parkinsons disease (PD) was PARK-1 a locus on chromosome 4q. This region contains the -synuclein gene in which two mutations (A30P and A53T) were shown to cause autosomal dominant PD. This protein was subsequently demonstrated to be a major component of Lewy bodies. We have been following a large family, the Iowan kindred with autosomal dominant disease ranging clinically from Dementia with Lewy bodies at one end of the spectrum to typical Parkinsons disease at the other. Recently, using the technique of quantitative PCR and in situ hybridization we have characterized a whole gene triplication event of the alpha synuclein gene. Furthermore, western blot analysis shows that this mutation results in overexpression of wild-type alpha synuclein protein in blood confirming the triplication event is responsible for the disease in this large kindred. We hereby report our results assaying for the gene triplication in a collection of 118 familial, 152 sporadic Parkinsons disease patients, and 92 controls using quantitative real time duplex PCR.

**A new quantitative PCR assay for rapid detection of aloid deletion of exon 7 in SMA health carriers.** A. Patitucci<sup>1</sup>, R. Mazzei<sup>1</sup>, FL. Conforti<sup>1</sup>, A. Magariello<sup>1</sup>, T. Sprovieri<sup>1</sup>, C. Rodolico<sup>2</sup>, AL. Gabriele<sup>1</sup>, G. Peluso<sup>1</sup>, G. Di Palma<sup>1</sup>, A. Toscano<sup>2</sup>, M. Muglia<sup>1</sup>. 1) ISN-CNR, Mangone, Cosenza, Italy; 2) Department of Neuroscience, Psychiatry and Anesthesiology Science, University of Messina, Italy.

The routine molecular test for diagnosis of SMA is based on the detection of a homozygous deletion of exons 7 and 8 of the telomeric copy of SMN gene. This does not allow the detection of the hemizygous absence of SMN1 gene which characterizes the carriers of the disease. The demand of SMN1 quantitative test is permanently growing because there is a high incidence of carrier frequency. Here we present a new non-radioactive quantitative PCR assay based on real time quantitative PCR. We analyzed 8 SMA patients, 14 related SMA patients and 50 normal control individuals by real time quantitative reaction using FAM labeled TaqMan MGB probe derived from SMN1 exon 7, and FAM labeled TaqMan probe with TAMRA quencher from RNase P gene as internal reference. SMN copy number was determined by the comparative threshold cycle method (Ct). The starting copy number of the unknown samples was determined comparing to the copy number of the calibrator sample applying the following formula:  $Ct = Ct_{RnaseP}(\text{calibrator sample}) - Ct_{SMN1-7}(\text{calibrator sample}) - [Ct_{RnaseP}(\text{unknown sample}) - Ct_{SMN1-7}(\text{unknown sample})]$ . The advantages of the real time quantitative PCR are two: first the possibility to calculate the gene copy number based on a Ct value, rather than using endpoint measurement; second it avoids the use of a standard curve with relative errors of dilution. The relative gene copy number was calculated by the expression  $2^{-Ct}$ . At first we determined genotype of SMA patients with traditional method of amplification and digestion with DraI for exon 7; then we analyzed all samples with this new quantitative method. The results confirmed the deletion in all homozygous patients and permitted to evaluate the number of alleles in the health carriers. This method is fast, reproducible, and makes us able to discriminate health subjects from health carriers, until now impossible with normal techniques like amplification and digestion.

**Diagnosis of haploid and triploid based on measurement of gene copy number in CMT and HNPP.** *T. Sprovieri, A. Patitucci, A. Magariello, FL. Conforti, R. Mazzei, AL. Gabriele, G. Peluso, G. Di Palma, M. Muglia.* ISN, CNR, Mangone, Cosenza, Italy.

A 1.5-Mb tandem duplication, including the gene for peripheral myelin protein 22 (PMP22) in chromosome 17p11.2-12 is responsible for 70% of the cases of Charcot-Marie-Tooth disease or hereditary motor and sensory neuropathy I (CMT1A/HMSN I). A reciprocal deletion of this CMT1A region causes the hereditary neuropathy with liability to pressure palsies (HNPP). We report a method for diagnosis of heterozygous deletions or duplications in HNPP and CMT based on measurement of gene copy number. This method involves amplifications of a test locus with unknown copy number and a reference locus with known copy number using real time PCR (ABI Prism 7900). The CMT1A duplication increases the PMP22 gene dosage from two to three, the HNPP deletion reduces the gene dosage from two to one. Our method is fast, reproducible and needs only a little quantity of DNA. We analyzed 41 CMT patients, 25 HNPP patients and 50 normal individuals by real time PCR using FAM labeled TaqMan MGB probe derived from PMP22 exon 4, and FAM labeled TaqMan probe with TAMRA quencher from RNase P gene as internal reference. Both reactions were run in parallel in the same time. The effective gene copy number was determined by the comparative threshold cycle method (Ct). The starting copy number of the unknown samples was determined comparing to the copy number of the calibrator sample applying the following formula:  $Ct = [Ct_{RnaseP}(\text{calibrator sample}) - Ct_{PMP4}(\text{calibrator sample})] - [Ct_{RnaseP}(\text{unknown sample}) - Ct_{PMP4}(\text{unknown sample})]$ . The relative gene copy number was calculated by the expression  $2^{-Ct}$ . The patients were previously examined by microsatellite analysis: most of patients showed the presence of three alleles, while a little number had two alleles with different dosage. The duplication in these last patients has been revealed by PFGE. The quantitative PCR confirmed the duplications in all examined patients. Thus, this method shows superior sensitivity to microsatellite analysis and has the additional advantage of being a fast and uniform assay for quantitative analysis of both CMT1A and HNPP.

**Comparison of a novel quantitative PCR assay with other methods of PMP22 gene dosage detection in HMSNI/HNPP.** *C. Thiel, C. Kraus, A. Rauch, A. Ekici, B. Rautenstrauss, A. Reis.* Institute of Human Genetics, University of Erlangen-Nuremberg, Germany.

A 1.4-Mb duplication including the gene for peripheral myelin protein 22 (PMP22) is responsible for 70 % of Charcot-Marie-Tooth disease 1A (HMSNI) while a deletion causes hereditary neuropathy with liability to pressure palsies (HNPP). Diagnosis is mainly performed with polymorphic markers (MS), fluorescence in situ hybridisation (FISH) and Southern hybridisation (SB). We have developed a novel single tube real-time PCR assay (qPCR) for determination of PMP22 gene dosage (Thiel et al. *Eur J Hum Genet* 2002) and now sought to evaluate the accuracy of this method through a retrospective analysis of 409 previously diagnosed patients (252MS, 157SB). Concurrent calling of the gene dosage was seen in 250/252 MS samples. One sample suspected of a deletion, gave normal results in qPCR and FISH analysis. One sample previously suggested unaffected by MS showed a duplication of the PMP22 gene in qPCR and FISH analysis. SB analysis in this sample using a PMP22 specific probe revealed a novel junction fragment due to an atypical, smaller duplication including PMP22. This confirms that MS analysis is a reliable method, but is prone to error in rare uninformative or atypical cases. Analysis of 157 patients previously diagnosed by SB revealed 12 inconsistencies (7,6%). In 8 cases a normal result was obtained with SB while qPCR diagnosed 5 duplications and 3 deletions. In 3 samples a difference in gene dosage was suspected but qPCR gave a normal result. MS analysis of all cases was concordant with qPCR results. In addition, one sample with a 7.8 kb junction fragment in SB showed normal gene dosage in qPCR, indicating the presence of a previously reported rare polymorphism. These findings confirm the lack of diagnostic precision of the absence/presence of junction fragments in SB. Our results show, that SB methods made 7% false callings while MS analysis was more reliable with a 1% false calling rate when compared to our new qPCR assay. We conclude that gene dosage detection in routine analysis of HMSNI and HNPP is most reliable when performed with this novel multiplex qPCR assay.

**FMR1 and FMR2 analysis in autistic population.** *T. Yamagata, K. Suwa, M. Mori, M. Nakamura, M.Y. Momoi.* Dept Pediatrics, Jichi Medical School, Tochigi, Japan.

FMR1 and FMR2 are genes responsible for fragile X syndrome (FRAXA) and FRAXE mental retardation (FRAXE), respectively. Both diseases are caused by the large CGG/CCG repeat expansion in 5'UTR of each gene that induce gene inactivation. Main symptoms of FRAXA are mental retardation (MR), typical facial appearance and macroorchidism. Some patients with FRAXA showed autistic symptom and about 0.75% (0-6%) of autistic population were reported to have CGG repeat expansion of FMR1. There is a report that no disease related base change was detected in FMR1 gene in autism patients. Most patients with FRAXE show mild MR or learning difficulty, but not facial abnormalities, and some patients were reported to have autistic phenotype. Thus far, there is no report about the repeat expansion or mutation screening of FMR2 gene in autistic population. Therefore, we screened FMR1 and FMR2 as candidate genes for repeat expansion and base substitution in Japanese autism population. We extracted DNA from patients with autism or pervasive developmental disorder not otherwise specified after obtaining informed consent from their parents. Repeat expansion of each gene was screened by PCR or Southern analysis. For mutation screening, each exon of the genes was amplified by PCR and mutations were screened by DHPLC method and sequencing. In FMR1 screening, one female autism patient had repeat expansion in 70 autistic patients. She was typical autism with mental retardation. In FMR2, no repeat expansion was detected. No base substitution that would relate to autism was detected in both FMR1 and FMR2, and several polymorphisms were detected. Our result on FMR1 analysis was same with previous report that FMR1 repeat expansion was the cause of autism in minor population. FMR2 repeat expansion was detected in only few patients in MR population and it might be same in autism population because we could not detect the mutation in autistic patients. Our sample was small and it should be analyzed further. However, most of the patients were caused by another genes mutations.

Program Nr: 1369 from 2003 ASHG Annual Meeting

**Translational Genetics in Cardiovascular Disease and Cancer; bridging the gap between basic research and clinical practice.** *J.C. Taylor.* Genetics Knowledge Park, Wellcome Trust Genetic Centre, Oxford, Oxfordshire, England.

The Oxford Genetics Knowledge Park (OxGKP) is part of a UK government-funded programme of translational clinical genetics. The major aim of the OxGKP is to ensure that advances in basic human genetics research are applied rapidly, efficiently and ethically in a health service framework. The OxGKP programme will focus on a few diseases which represent the spectrum of genetic complexity and can be used as paradigms for future studies. We are studying disorders such as sudden cardiac death (SCD), which result from multiple Mendelian variants, through to more complex diseases such as cardiovascular disease and cancer, where multiple DNA variants and environmental factors contribute to the end phenotype. For SCD we will use Wave DHPLC to identify mutations in the proband and sequencing to identify other family members at risk. For cardiovascular disease (CVD), mass spectrometry will be used to genotype polymorphisms in candidate genes in large cohorts. Variants associated with susceptibility to CVD or response to statins may be identified using these cohorts. Microarray-based comparative genomic hybridisation (CGH) will be developed for detection of somatic mutations in colorectal cancer tissues and identification of variants which correlate with response to chemotherapy. This technique will potentially provide an efficient and cost-effective alternative to classical cytogenetics for detecting chromosomal deletions and duplications in many other disorders. In addition to addressing the scientific and technical issues which must be resolved before such genetic tests can be offered, the OxGKP programme will assess the ethical, legal and sociological implications of providing such tests. Finally, we will calculate whether the introduction of expanded genetic testing services would be a cost-effective initiative for health services. Like other aspects of biomedicine, there is a gap between new discoveries and their implementation in clinical practice that is often difficult to bridge. This programme should considerably facilitate this transition in the area of human genetics.

**Cost-effectiveness of DNA based screening for Familial Medullary Thyroid Carcinoma.** *J.L. Brown<sup>1</sup>, P.J. Bridge<sup>2</sup>, D.W. Morrish<sup>3</sup>, D.M. Gilchrist<sup>3</sup>*. 1) Medical Genetics Clinic, University of Alberta Hospital, Edmonton, AB, Canada; 2) Department of Medical Genetics, University of Calgary, Calgary, AB, Canada; 3) Department of Medicine, University of Alberta, Edmonton, AB, Canada.

**Objective:** One of the goals of genetic testing is to reduce morbidity and mortality. A secondary goal is reduction in health care costs. This can be demonstrated most effectively in large families with mutations in dominantly acting genes. **Methods:** A point mutation (Cys620Arg) in exon 10 of the RET proto-oncogene was first identified in two individuals in a large French Canadian family. The proband had medullary thyroid carcinoma (MTC), as did her deceased mother. One son has MTC and Hirshsprung. The proband's mother had nine siblings; the proband has three siblings, another son, and 69 maternal first cousins. Genetic testing has commenced with closest relatives. **Results:** Five out of six living siblings of the proband's mother have tested negative for the mutation. This has eliminated the need for genetic testing in 36 cousins. One of the proband's sisters tested positive and both her children tested negative. Four nieces and nephews did not require genetic testing because of negative parental status. This has saved \$4,800 in genetic testing (\$120 per test). Clinical screening for individuals at risk for familial MTC involves annual pentagastrin screening (\$279 yearly). Clinical savings in one year for the 49 excluded family members are \$13,671. The savings for MEN2 screening would be even more significant. **Conclusion:** Economic costs of genetic diagnostics should take into account potential saved monies in tests, both molecular and clinical, for relatives. Note: 1 Canadian Dollar = 0.74873 US Dollar.

Program Nr: 1371 from 2003 ASHG Annual Meeting

**Development and use of risk assessment criteria to evaluate cancer family histories.** *H. Hampel, K. Sweet, J.A. Westman, C. Eng.* Clinical Cancer Genetics Program, Division of Human Genetics, The Ohio State University, Columbus, OH.

Assessing a cancer family history is a powerful public health tool for stratifying patients according to their risk of developing cancer so that they can be managed appropriately. A standard set of risk assessment criteria is needed to ensure consistency when different individuals or institutions are assessing risk. The objective of this study was to develop criteria to assist in comprehensive and consistent risk assessment of cancer family histories. The criteria are based on comprehensive literature review of publications describing diagnostic criteria for hereditary cancer syndromes and risk to first-degree relatives of cancer patients, with priority given to consensus statements. These criteria were used to assess 2828 cancer family histories obtained from an ambulatory patient area at a comprehensive cancer center (CCC). These criteria categorized 339 (12%) families into the High Risk level leading to referral for cancer genetics consultation and a recommendation to discuss increased cancer surveillance with their primary care physicians. Another 450 (15.9%) fulfilled at least one Moderate Risk criterion, a risk level that does not require referral for cancer genetics consultation but does suggest discussion of increased cancer surveillance with their physicians. Most families (69.5%) were categorized as Low Risk, i.e., a similar cancer risk to the general population, as expected. The remaining 85 (3%) users did not meet High or Moderate Risk criteria, but were offered cancer genetic consultation because of a concerning family history. This set of cancer risk assessment criteria successfully stratifies the expected proportion of patients into the various risk categories, and are easy to apply to large-scale family history screening.

**Breast cancer genetic susceptibility: does testing first an affected family member impose an undue burden?** *P. Rowley, C. Shields, S. Loader.* Univ Rochester Medical Ctr, Rochester, NY.

In cancer genetic risk counseling, the standard practice is to test an affected family member first. Typically the person seeking testing is unaffected and must therefore recruit a relative with cancer to be tested. Does this practice impose an undue burden on the recruited relative?

We have previously reported a clinical trial in which any woman in a defined geographic region who had a qualifying family history and who was referred by her physician was offered free genetic counseling and DNA testing. (Loader et al. 1998). The index family member could have breast or ovarian cancer (affected) or not (unaffected or at risk), but had to recruit another family member willing to be tested so that each family was represented by one affected and one unaffected person. Additional patients were recruited from a cancer registry. Of 140 women qualifying, 112 came for pretest education. After pretest education, 98 chose to be tested; of 87 families actually tested, thirteen had deleterious mutations.

To assess the impact of the counseling and testing process, we recontacted the individuals at one month and at one year and those with abnormal test results at four years following the communication of the test result. We found that index subjects differed significantly from relatives. Before coming for counseling, index subjects, whether having had cancer or not, perceived both their general health and emotional health as worse than did relatives. After counseling and testing, index subjects continued to worry more about breast cancer than did relatives.

In summary, the group most prone to distress by cancer risk genetic counseling and testing is, not the recruited relatives, nor even those affected with cancer, but rather the index patients themselves. Thus relatives do not appear to be unduly burdened by the process. The index patients, i.e. the ones who want the risk information most, appear to undergo the most stress in obtaining it.

**Meeting a Need: Genetic Cancer Risk Assessment Protocol for an Underserved Population.** *C. Ricker<sup>1</sup>, V. Lago<sup>1</sup>, S. Fuentes<sup>2</sup>, S. Hiyama<sup>3</sup>, V. Kumar<sup>2</sup>, N. Feldman<sup>3</sup>, G. Uman<sup>4</sup>, K. Blazer<sup>1</sup>, J. Weitzel<sup>1</sup>.* 1) Clinical Cancer Genetics, City of Hope Natl Medical Ctr, Duarte, CA; 2) QueensCare Foundation (QC), LA CA; 3) OliveView Medical Center (OV), Sylmar CA; 4) Vital Research, LA CA.

Outreach clinics were established at OliveView Medical Center (OV), a county indigent care hospital, and QueensCare (QC), a foundation-funded clinic for individuals at < 200% of poverty level, to provide genetic cancer risk assessment (GCRA) to underserved (predominately Latino) patients. A needs assessment survey on knowledge and perceptions about cancer and screening, administered in QC and OV general medicine and oncology waiting rooms, documented gaps in knowledge and interest in prevention, indicating a need for and receptivity to GCRA. Thirty-five percent of respondents had a personal and/or family history of cancer and 80% voluntarily included an address to receive more information. Clinicians at both sites participated in CME lectures on cancer genetic and referral guidelines were disseminated and posted. A marked increase in clinician knowledge (66-72%) was demonstrated and their understanding was reflected by the quality of referrals, of which 85% were appropriate. Patients participated in language appropriate GCRA. Each of the 14 families found to carry deleterious mutations had on average 23 at-risk family members (range 10-32). This extension of GCRA impact to other family members is referred to as the Family Amplification Factor (FAF). Feasibility and acceptability of the program was demonstrated by the high rate of clinic attendance (88% of patients scheduled kept appointments), supporting our hypothesis that GCRA would be well received. Future impact was foreshadowed by 4 mutation carriers with bilateral breast cancer, who would have been candidates for genetic testing at the time of their first diagnoses. Had GCRA services been accessible, their second diagnosis might have been avoided or detected at an earlier, more treatable stage. Preliminary findings indicate that investigation of follow-up care uptake is necessary to judge the efficacy of GCRA with respect to cancer risk reduction. However, indigent patients are motivated and as likely to benefit, as insured patients.

**Novel Mutation L187P in KCNQ1 Causes Autosomal Dominant Long QT Syndrome.** *M. Liu*<sup>1,2</sup>, *L. Zhang*<sup>3</sup>, *L. Wang*<sup>2</sup>, *S. Chen*<sup>2</sup>, *G.M. Vincent*<sup>3</sup>, *Q. Wang*<sup>2</sup>. 1) Institute of Genetics, Fudan University, Shanghai, Shanghai, P. R. China; 2) Center for Molecular Genetics, Department of Molecular Cardiology, Lerner Research Institute, and Center for Cardiovascular Genetics, The Cleveland Clinic Foundation, Cleveland, OH 44195; 3) Department of Internal Medicine, LDS Hospital and University of Utah Health Sciences Center, Salt Lake City, UT, 84103.

Congenital long QT syndrome (LQTS) is characterized by a prolonged QT interval and polymorphic ventricular arrhythmias (torsade de pointes) and sudden death. Here we report a novel potassium channel KCNQ1 mutation in a large autosomal dominant family. Methods: A 7-generation family pedigree was established to search affected members by electrocardiogram (ECG) screening. Forty-two family members agreed to participate in the genetic study. Five markers (D11S4046, D7S798, D3S1277, D4S402, and D21S266) were used for linkage analysis. Results: The disease gene of this family is linked to D11S4046 with a lod score of 6.11 ( $q=0.10$ ). SSCP and sequence analysis in the coding region of KCNQ1 gene (including exon-intron boundaries) identified SNP T-C transversion at codon 187 (between transmembrane domains S2 and S3), which is predicted to result in a substitution of proline for leucine (L187P). SSCP and RFLP analysis showed that mutation L187P co-segregated with the disease. Thirty-one family members were identified as mutation carriers (age 3321 yrs, 13F) with mean QTc 0.460.03 sec and 12 non-carriers (age 3022 yrs, 4F) with mean QTc 0.430.02 sec. Typical LQT1 T wave pattern were present in 84% of gene carriers. 16% were symptomatic. There was one sudden death before LQTS diagnosis. 58% (18/31) of carriers had normal to borderline QTc (0.450.04 sec) in the initial ECGs. RFLP analysis was performed in 200 normal controls, but none of them carry the mutation. Conclusions: L187P causes low QTc penetrance in this LQT1 family, highlighting the importance of genetic testing.

**High-throughput genotyping of recurrent mutations in hypertrophic cardiomyopathy by MALDI-TOF mass**

**spectrometry.** *C. Wei*<sup>1</sup>, *A. Romaschin*<sup>1</sup>, *W. Newman*<sup>2</sup>, *D.E.C. Cole*<sup>3</sup>, *K. Siminovitch*<sup>2</sup>, *M. Yazdanpanah*<sup>2</sup>. 1) Molecular Genetics Laboratory, Toronto Medical Laboratories, Toronto, ON, M5T 1V4, Canada; 2) Analytical Genetics Technology Center, Department of Medicine, Princess Margaret Hospital/University Health Network, Toronto, ON, M5G 2L7, Canada; 3) Departments of Laboratory Medicine & Pathology, Medicine, Paediatrics/Genetics, University of Toronto, Toronto, ON, M5G 1L5, Canada.

Familial hypertrophic cardiomyopathy (HCM) is an autosomal dominant myocardial disorder with incomplete penetrance and variable clinical expression. Clinical findings include left ventricular hypertrophy, dysrhythmias, heart failure and sudden death. More than 200 mutations in 10 genes encoding mainly sarcomeric proteins have been reported. Molecular diagnosis may lead to better understanding of genotype-phenotype correlations and better clinical management. However, it is technically challenging to screen for recurrent mutations that represent a minority of the total number of potential mutations. The University Health Network provides clinical care for one of the worlds largest cohorts of HCM, with more than 600 families. To facilitate mutation detection in this group, we have initiated a highthroughput protocol for known mutations using the Sequenom Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) Mass Spectrometer. Automated genotype call efficiency was 95% and assays precision and accuracy were 99.9%. Based on this technology, we developed a multiplex PCR for six mutations including P211L, R403Q, R403L, and L908V in the MYH7 gene, and P76L, and IVS15+1G A in the TNNT2 gene. While the great majority of samples were negative for all 6 mutations, MYH7 L908V was correctly identified in two samples from the same pedigree and MYH7 R403Q in a third sample. The two positives for the L908V mutation were confirmed by PCR and RFLP analysis. Based on this experience, MALDI-TOF has considerable promise as a high-throughput means of screening for HCM mutations with a prevalence of no more than 1-2%. We are currently setting up three more multiplex PCR reactions for a total of fourteen known mutations in MYH7, TNNT2, TNNI3, MYL2 and MYL3 genes.

**Presymptomatic Diagnosis of Inherited Disorders: Classification of Medical Interventions.** C. Dolan<sup>1,2</sup>, K. Marymee<sup>3</sup>, A. Amemiya<sup>2</sup>, J. Edwards<sup>1</sup>, I. Saphire-Bernstein<sup>4</sup>, P. Tarczy-Hornoch<sup>1</sup>, R. Pagon<sup>1,2</sup>. 1) University of Washington, Seattle; 2) Children's Hospital and Regional Medical Center, Seattle; 3) Inland Northwest Genetics Clinic, Spokane; 4) Blue Cross Blue Shield Association, Chicago.

**SIGNIFICANCE:** Presymptomatic diagnosis of inherited disorders in at-risk individuals has clinical utility when early diagnosis permits interventions that reduce morbidity and/or mortality.

**BACKGROUND:** While creating "GeneNotes," one-page summaries of genetic counseling and testing basics based on *GeneReview* entries ([www.genetests.org](http://www.genetests.org)), we recognized the need to classify the types of medical interventions made possible by early diagnosis of at-risk individuals.

**METHODS:** GeneTests staff reviewed medical interventions for the 72 diseases in *GeneReviews* for which early diagnosis of an at-risk asymptomatic individual alters medical management.

**RESULTS:** The four classes of medical interventions and examples include: 1) Treatment to prevent primary disease manifestations (23 diseases): e.g., PKU (dietary restriction), Wilson disease (copper chelation); 2) Treatment to prevent secondary complications (15 diseases): e.g., sickle cell disease (penicillin administration), hearing loss/deafness (hearing aids/communication skills); 3) Surveillance (30 diseases): e.g., hereditary cancer syndromes, Stickler syndrome (retinal detachment); 4) Avoidance (22 diseases): e.g., Romano-Ward syndrome (medications that prolong QT interval, hypohidrotic ectodermal dysplasia (fever).

**CONCLUSIONS:** Clarification of the medical benefits of early diagnosis of at-risk individuals through presymptomatic genetic testing may help establish clinical utility and improve reimbursement of clinically appropriate testing costs by third-party payers, thereby increasing patient access to testing. Discussions initiated with the Blue Cross and Blue Shield Association will explore the usefulness of this classification system.

**Detection of the 22q11.2 deletion within a population containing Specific Language Impairment (SLI) subjects.**

*D.R. Collins*<sup>1, 2</sup>, *J.C. Murray*<sup>2</sup>, *J.B. Tomblin*<sup>1</sup>, *S.A. Bullard*<sup>3</sup>. 1) Department of Speech Pathology and Audiology, University of Iowa; 2) Department of Pediatrics, University of Iowa; 3) Dows Institute of Dental Research, University of Iowa.

The purpose of this investigation was to screen for the 22q11.2 deletion associated with Velocardiofacial Syndrome (VCFS) in a population of subjects containing 121 normal controls, 56 individuals with Specific Language Impairment (SLI), 62 individuals with low nonverbal intelligence quotients but normal language abilities, and 38 subjects with below normal language abilities and low nonverbal intelligence quotients. Subjects were tested for type of language disorder by using components from the following diagnostic battery of tests; CELF-3, WISC-3, PPVT-3, and Woodcock Johnson. A diagnosis of SLI required a nonverbal IQ above 85 and failure on two or more language composite tests with a z score of 1.25 or less. In total, 277 subjects were successfully screened for the 22q11.2 deletion using a real time polymerase chain reaction protocol adapted from Kariyazono et al (2001). The results of this investigation indicated that one of the SLI subjects was identified as potentially having the 22q11.2 deletion. Additionally, after a review of the subjects medical records, it was also noted that this individual had been seen in cleft palate clinic for persistent hypernasality and language delay. The discovery of the 22q11.2 deletion within the SLI population has important clinical implications in regard to the potential genetic source of SLI and the incidence of VCFS within this population. The SLI population is considered to be a very heterogeneous and the genetic cause of SLI is considered to be multifactorial. According to the results of this study, it is possible that individuals with VCFS may display the language phenotype of SLI and that the incidence of VCFS may be higher within SLI population as compared to the normal population. However, the identification of an individual diagnosed with both VCFS and SLI remains a rare finding.

**Immunoblotting of ATM Protein and Kinase Activity for Diagnosing A-T.** S.A. Nahas, H.H. Chun, R.A. Gatti.  
Department of Pathology, UCLA School of Medicine, Los Angeles, CA 90095.

The laboratory diagnosis of ataxia-telangiectasia (A-T) currently relies upon measurement of serum alphafetoprotein (AFP) and cellular sensitivity to ionizing radiation. A previous report suggests that immunoblotting of whole cell lysates from lymphoblastoid cell lines (LCLs) might be informative for diagnosis. To further evaluate this possibility, and improve sensitivity, we performed immunoblotting for ATM protein on nuclear lysates of 71 consecutive radiosensitive LCLs that were established from patients with clinical features suggestive of A-T. Fifty-two LCLs (73%) contained no detectable ATM protein, with a representative sample (n=25) testing negative for ATM kinase activity, having at least one ATM mutation, and having elevated AFP levels; these results confirmed the diagnosis. Seventeen LCLs (24%) expressed intermediate or normal levels of ATM protein and exhibited normal ATM kinase activity; follow-up studies failed to detect ATM mutations and AFP levels were normal in all but three. Of the remaining two radiosensitive LCLs, one had 35% of normal protein with normal kinase activity and no ATM mutations (AFP was borderline). The other LCL had 9% of normal protein, with intermediate levels of kinase activity, homozygous missense ATM mutations and an elevated AFP. Our data suggest that it is very uncommon to encounter bonafide A-T patients with more than trace amounts of ATM protein in nuclear lysates. We conclude that immunoblotting for ATM protein and kinase activity is of higher specificity for diagnosing A-T than radiosensitivity testing. In addition, we have documented *in vitro* radiosensitivity in other patients who share some clinical features with A-T but must have some other disorder(s).

**Diagnostic strategy for Prader-Willi syndrome and Angelman syndrome in Korea.** *S.M. Ahn<sup>1</sup>, G.H. Kim<sup>1</sup>, E.Y. Choi<sup>1</sup>, S.H. Heo<sup>1</sup>, J.Y. Lee<sup>1</sup>, J.S. Kim<sup>1</sup>, H.W. Yoo<sup>1,3</sup>, E.J. Seo<sup>1,2</sup>.* 1) Medical Genetics & Lab., Asan Medical Center, Seoul, Korea; 2) Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea; 3) Department of Pediatrics, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.

Prader-Willi syndrome(PWS) and Angelman syndrome(AS) are clinically different neurobehavioral disorders resulting from molecular defects of 15 chromosome originated from father or mother. These disorders can result from microdeletion, uniparental disomy(UPD), or imprinting center defect in specific region 15q11-13 of chromosome. We had studied G-banding, FISH and methylation-specific PCR (MS-PCR ) for 96 patients with clinical features suggestive of PWS and AS. Microsatellite analysis was done in doubtful patients of UPD and mutation of UBE3A gene was analyzed in patients referred to AS. Of 85 patients referred for PWS, 24 patients had only maternal pattern by MS-PCR and were confirmed to PWS. In 21 of 24 confirmed PWS patients were found to show deletions by G-banding and FISH and 3 patients detected to deletion only by FISH. The remaining 3 patients has maternal UPD. In AS cases, 5 patients were suspected of having AS after detection of deletions by G-banding, and then confirmed by FISH and MS-PCR. Of 6 patients referred to AS tests by physician, 2 patients had deletion and one patient had single base deletion in exon 9 of UBE3A. Twelve patients of 64 patients with normal methylation pattern have shown abnormal karyotyping results exception with chromosome 15. These results show that PWS and AS are disposed to be misdiagnosed or underdiagnosed because many features are nonspecific or subtle. Therefore we recommend a effective strategy for diagnostic testing of PWS and AS. First of all, G-banding should be performed for all patients suspected of PWS and AS. In Korea, cost of cytogenetic analysis is from one-third to a half of one of FISH or MS-PCR. Simultaneously, MS-PCR must be analysed to diagnose accurately PWS and AS, followed by FISH or microsatellite analysis. In cases with normal methylation pattern and strongly suspected of PWS and AS, tests for imprinted genes can be performed lastly.

**Detecting chromosomal aberrations within 6-8 hours using MLPA.** *S.J. White, M. Kriek, M.E. Kalf, G-J.B. van Ommen, M.H. Breuning, J.T. den Dunnen.* Human and Clinical Genetics, Leiden University Medical Center, The Netherlands.

Genomic deletions and duplications play an important role in the etiology of human disease. It is therefore essential that the tests necessary to detect such rearrangements are available and readily applicable in diagnostic laboratories. Multiplex Ligation-dependent Probe Amplification (MLPA) is a technique that allows the rapid and precise quantitation of multiple (>40) sequences within a nucleic acid sample. It is based upon the hybridization, ligation and PCR amplification of 2 oligonucleotides. Common ends on the oligonucleotides mean that amplification can be performed with one primer pair. We are currently examining several different applications of this technique within a clinical setting. One use is for aneusomy screening of new borns. In cases where a diagnosis is uncertain it is often necessary to quickly rule out specific chromosomal abnormalities e.g. trisomy chromosome 13 or 18. The confirmation of a specific microdeletion/duplication syndrome may also be of benefit. To facilitate this we have performed the MLPA reaction on blood spotted onto an FTA card (Whatman). This virtually eliminates preprocessing, meaning that it is possible to go from blood sample to result within 6-8 hours. Using this method we have detected whole chromosome trisomies, as well as cytogenetically invisible rearrangements. The high resolution potential has prompted us to explore the possibility of using this as an alternative to FISH. The resultant PCR products have been successfully analyzed in several different ways, including on a capillary sequencer, the Lab-on-a-chip (Agilent) or by hybridization to a microarray. We are currently testing the possibility of simultaneous analysis of several hundred probes using arrays, providing a resolution far greater than that obtainable with FISH or array CGH. The fact that any combination of probes can be easily combined in a single reaction, coupled with the speed and flexibility of analysis afforded by the different platforms means that this technique has an important role to play in molecular cytogenetics within a diagnostic setting.

**Validation studies for Noonan syndrome molecular diagnostic testing.** *J. Wiszniewska<sup>1</sup>, M. Maheshwari<sup>1</sup>, S. Fernbach<sup>1</sup>, J. Belmont<sup>1, 2</sup>, B.B. Roa<sup>1</sup>.* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pediatrics, Baylor College of Medicine, Houston, TX.

Noonan syndrome is an autosomal dominant, genetically heterogeneous disorder with an estimated prevalence of approximately 1 in 1,000-2,500. Characteristic features include hypertelorism, downslanted eyes, posteriorly rotated low set ears, short stature, short webbed neck, cardiac anomalies, motor delay, and a bleeding diathesis. Approximately 50% of Noonan syndrome cases are associated with mutations in the PTPN11 gene, which maps to 12q24.1 and encodes the non receptor protein tyrosine phosphatase SHP-2. Patients with PTPN11 gene mutations are considered to have a higher prevalence of pulmonic stenosis as a cardiac manifestation. To date, mutations identified in the PTPN11 gene are missense alleles that are mostly clustered in N terminal SH2 and PTP domains. We developed an assay for routine molecular diagnostics of Noonan syndrome that involves bi-directional sequencing analysis of 15 coding exons of the PTPN11 gene. Validation studies were completed on 12 blinded DNA samples, including mutation-positive patients who were previously diagnosed with Noonan syndrome at our institution. DNA samples from seven Noonan syndrome patients were correctly identified to have the following documented PTPN11 missense mutations: Y62D, Y63C, F285L, S502T, and M504V. Additionally, sequence polymorphisms in intron 7 were also identified. Diagnostic sequencing analysis of PTPN11 gene is currently being offered for Noonan syndrome at the Baylor DNA Diagnostic laboratory. Data on molecular testing will be collected prospectively to determine the mutation detection rate in a clinical diagnostic setting, and hopefully to refine genotype-phenotype correlations for PTPN11 mutations in Noonan syndrome.

**An automated platform for the identification of fetal cells in maternal circulation.** *F. Tafas<sup>1</sup>, M.W. Kilpatrick<sup>1</sup>, I. Ichetovkin<sup>1</sup>, P. Rompolas<sup>1</sup>, A. Seppo<sup>1</sup>, Y. Agarwal<sup>1</sup>, Y. Kim<sup>1</sup>, J. Recht<sup>1</sup>, L. Strinkovsky<sup>1</sup>, A. Antsaklis<sup>2</sup>, B. Brambati<sup>3</sup>, P. Tsipouras<sup>1</sup>.* 1) Ikonisys Inc, New Haven, CT; 2) University of Athens, Greece; 3) University of Milan, Italy.

The presence of rare fetal cells in the maternal circulation is generally accepted, but their number, timing of appearance, and the best approach for their enrichment remain unclear. Fetal cells can be indisputably identified through detection of paternal genetic material, but over a million cells per maternal sample must be analyzed for detection of a handful of cells containing Y FISH signals. We developed an automated system to scan for cells bearing X and Y FISH signals. Target cells are screened at 20X magnification then verified at 100X. 20X images of all signals in each of 4,000 optical fields per slide and 100X images of target cells are displayed. We initially used single X and Y FISH probes but identified cells bearing single X and Y signals in all 12 maternal samples scanned. XY cells were indistinguishable, at both 20X and 100X, regardless of fetal sex, suggesting that FISH can cause a significant level of false positive signals affecting the specificity of automated chromosome counting. We therefore incorporated two independent Y-chromosome probes; one centromeric and one telomeric. Putative fetal cells are identified at 20X magnification based on the presence of the X and telomeric-Y signals, and fetal cells verified at 100X magnification based on the presence of a single X-chromosome signal, and single signals for each of the centromeric and telomeric Y-probes. Implementation of this approach reduced the false positive rate for detection of XY nuclei below 0.00005%. In 7 samples from pregnancies with male fetuses the automated system identified 1 - 7 XY cells per ml maternal blood; no XY cells were found in 5 samples from female fetus pregnancies. Ikonisys has developed a high fidelity automated system for the quantitation of fetal cells in the maternal circulation. It is capable of highly accurate detection and quantitation of FISH signals and has potential applications in a variety of areas where the reliability and speed offered by an automated system would be of benefit.

**Novel Approach to Improved Access to Cancer Genetics Services: the Columbus, Ohio Solution.** *M. Goulet<sup>1</sup>, L. Ahonen<sup>2</sup>, H. Hampel<sup>3</sup>, R. Nagy<sup>3</sup>, K. Sweet<sup>3</sup>, L. Vassy<sup>1</sup>, B. Sickel-Santanello<sup>2</sup>, C. Eng<sup>3</sup>.* 1) Cancer Risk Program, Mount Carmel Health, Columbus, OH; 2) Cancer Genetics Program, OhioHealth, Columbus, OH; 3) Human Cancer Genetics Program, Ohio State Univ.(OSU),Columbus, OH.

By developing strategic partnerships with a 6 year old cancer genetics program, patients have increased access to cancer risk assessment and genetic counseling services. Mount Carmel (in August 2001) and OhioHealth (in August 2002), both community hospital systems, entered into a collaborative relationship with OSU's academic medical center. This model allows patients to remain within their own healthcare systems and under their referring physicians' continued care while utilizing the geneticist and genetic counseling expertise already in place at OSU. Cancer genetics services are provided without the economic disadvantage of duplicating existing services within the community. This city-wide partnership has increased the number and diversity of referring physicians while decreasing the number of patients receiving genetic testing without appropriate counseling. Of the 97 physicians who referred patients to the community hospitals' cancer genetics programs, 78 physicians had not previously referred to OSU's cancer genetics program. Twelve area physicians had ordered BRCA gene testing directly from their office without genetic counseling prior to the partnerships; only 1 physician has since continued that practice. The collaboration has also afforded patients the opportunity to participate in numerous research studies available throughout the country. This solution demonstrates that the efficiency and effectiveness of a program is sometimes best achieved when competitors become collaborators and place more emphasis on patient, physician, and staff needs.

**Insight into genetic testing for non-disease traits: a qualitative assessment.** *E.S. Gordon<sup>1</sup>, H.A. Gordish-Dressman<sup>1</sup>, J.M. Devaney<sup>1</sup>, P.M. Clarkson<sup>2</sup>, M.J. Hubal<sup>2</sup>, P.M. Gordon<sup>3</sup>, E.E. Pistilli<sup>3</sup>, L.S. Pescatello<sup>4</sup>, B. Kelsey<sup>4</sup>, G. Gianetti<sup>4</sup>, E.P. Hoffman<sup>1</sup>.* 1) Res Ctr Gen Medicine, Children's National Med Ctr, Washington, DC; 2) Dept of Exercise Science, Univ of Massachusetts, Amherst, MA; 3) Div of Exercise Physiology, West Virginia Univ, Morgantown, WV; 4) Health Promotion and Allied Health Sciences Dept, Univ of Connecticut, Storrs, CT.

As the field of genetics evolves from its focus on single gene disorders to complex disorders and normal traits, we must consider the psychological impact of this evolution. Here we present data from a qualitative assessment of 105 participants insights into non-disease genetic testing. All participants were part of a larger four year study (Factors affecting muscle size and strength; FAMuSS). The long-term goal of this study is to assess the impact of non-disease genetic information on participants, including their views on non-disease genetic testing for themselves, their children, concerns about insurance and job discrimination and the direct impact that non-disease genetic testing may have on their lives. At the time of enrollment (T1), assessment tools including a series of qualitative, open-ended questions as well as standardized assessments of self-concept (Tennessee Self-Concept Scale; Health Orientation Scale) were administered to the participant. Each participant donated a blood sample for genotyping of 4 SNPs previously shown to influence muscle performance: ACE, CNTF, gamma sarcoglycan and UPC-2 genes. After completing a 12 week exercise program, participants were told which SNPs they possess and were given information about the traits associated with each SNP. Both qualitative and standardized assessments were then repeated (T2). In a comparison of responses prior to and after receiving results related to the ability to gain muscle strength and/or muscle mass, only 2% of participants felt that the information changed their self-concept. However, 34% of participants reported that the information they received would change their behavior. This is a clear indication of the impact of non-disease genetic testing and the realization that caution must be taken when providing this information.

**The contribution of polymorphisms of the TNF gene to disease severity, susceptibility to amyloidosis and response to treatment in patients with familial Mediterranean fever.** *R. Gershoni-Baruch<sup>1</sup>, R. Brik<sup>1</sup>, N. Zaks<sup>2</sup>, M. Lidar<sup>2</sup>, M. Shinawi<sup>1</sup>, A. Livneh<sup>2</sup>.* 1) Dept Human Genetics and pediatrics, Rambam Medical Ctr, Haifa, Israel; 2) Heller Institute of Medical Research, Sheba Medical Center, Tel-Hashomer, Israel.

Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by attacks of fever and serositis, and a predisposition to the development of amyloidosis. The disease is noted for its wide clinical variability, which is mainly, yet not solely, attributed to MEFV allelic heterogeneity. Modifiers contributing to the phenotypic profile in FMF remain to be defined. In this study we investigated the effect of TNF-alpha promoter polymorphisms, which seem to be related to an individual's production of TNF-alpha, on disease severity, susceptibility to amyloidosis, and response to treatment, in 150 FMF patients, who were all homozygous for the M694V mutation. A detailed chart review and physical examination were undertaken to determine demographic characteristics, history, clinical manifestations, treatment, and calculate the severity score from the Tel-Hashomer key. Using sequence-specific primers to the TNF-alpha gene and polymerase chain reaction, we examined three single nucleotide polymorphisms (SNPs) of the TNF-alpha gene, TNF (-376G/A, 308 G/A and -238 G/A). An association was sought between the presence of high-producing (A homozygotes, GA heterozygotes) or low-producing (G homozygotes) TNF-alpha genotype with disease severity, the development of amyloidosis and response to treatment. This results of this study suggest that the TNF promoter polymorphisms do not significantly influence the patients' outcome and are not positively associated with amyloidosis or response to treatment. The presence, however, of the high-producing TNF-alpha alleles led to an earlier age of onset, yet the difference was not significant. Further investigation is required to determine if these polymorphisms or others (in the TNF-receptor genes) play a key role in the pathogenesis of FMF.

Program Nr: 1386 from 2003 ASHG Annual Meeting

**Medical challenges of practicing medicine through the approach of principles of genomic science of systems biology.** *R. Chakraborty*. Dept. of Environmental Health, Univ. Cincinnati, Cincinnati, OH.

The emerging field of systems biology as a discipline of studying complexity of biological systems and its biomedical applications has a direct relevance on using the tools of functional genomics from laboratory to the patients. Advocates of this *-omics* platform of practicing medicine claim that this approach would be evidence-based, through which patients should be able to receive personalized attention for treatment, control, as well as prevention. It is true that the systems-based approach of understanding diseases may circumvent some of the reductionism-related drawbacks of contemporary medicine. However, new medical challenges that would arise from such post-genomic approaches of practicing medicine are not trivial, and policies to combat such challenges are not exactly in place. This presentation raises some of these issues, and offers suggestions for development of guidelines for meeting these challenges, based on models of other post-genomic translational research. Need for international enforcement of such guidelines is also important in this regard. Apart from the privacy and safety issues in relation to the information gathered, which can be raised by the patients, providers of post-genomic medicine will have to make patients aware of uncertainties of risk estimation, predictions about range of outcomes of treatment modalities, and side effects, based upon which the patients are to be counseled with regard to options open to them and their cost-benefit analyses. Since the providers of post-genomic medicine will consist of teams with contributions from academia, industry, venture capitalists, health care providers, as well as governmental and legal agencies, guidelines for meeting such challenges and protocols for enforcing them are not so trivial. Patent laws and rules of accessibility of databases may also need to be revisited in this context. But it is not too early to formulate an integrative set of guidelines to institutionalize individualized medicine based on the advances of being made in this field of translational research. (Supported by US Public Health Service Research grants from the National Institutes of Health).

**Impact of Genetic Cancer Risk Assessment (GCRA) in an Underserved Setting: Outcomes and Adherence to Cancer Risk Management Recommendations.** *V. Lagos<sup>1</sup>, C. Ricker<sup>1</sup>, S. Fuentes<sup>2</sup>, S. Hiyama<sup>3</sup>, V. Kumar<sup>2</sup>, N. Feldman<sup>3</sup>, J. Weitzel<sup>1</sup>.* 1) Clinical Cancer Genetics, City of Hope, Natl Medical Ctr, Duarte, CA; 2) QueensCare Foundation, Los Angeles, CA; 3) OliveView Medical Center, Sylmar, CA.

Outreach clinics were established with community collaborators to address populations that are underserved by virtue of socioeconomic status as well as by lack of access to genetic cancer risk assessment (GCRA). The objective is to determine impact of GCRA on access, adherence and barriers to cancer screening and prevention recommendations. Patients referred for GCRA were consented and enrolled in a hereditary cancer registry. A knowledge test covering genetic principles, hereditary risks, and risk reducing intervention was administered immediately post-GCRA to assess initial learning and at 12 months to assess knowledge retention. Adherence to risk management recommendations was assessed by structured interviews at six months and one year post-GCRA. The study population (n=50) was predominantly Hispanic (75%). Fifty-eight percent had a current or past diagnosis of cancer and the average gene mutation probability was 28%. Thirty percent of patients proceeded with testing and 14 families were found to be carriers of a deleterious mutation. Follow-up has been conducted on 37 patients. The mean score on the post-GCRA knowledge survey was 78%. Behavioral impact was exemplified by the fact that all patients interviewed six months post-GCRA were performing monthly breast self-exams and all have been able to obtain a clinical breast exam. Seventy one percent of the women whose recommendations included a mammogram within the follow-up period have had one. Every patient interviewed has expressed her satisfaction with the GCRA experience. Other outcomes include expressions of reduced anxiety, increased understanding of cancer and personal risk, and better grasp of care options. Based on preliminary data GCRA had a positive impact on these patients and they appear motivated to adhere to cancer risk management recommendations. Longer term follow-up on a larger sample is necessary to determine the uptake of risk reduction surgery and to characterize personal and healthcare system barriers to follow-up care.

**Establishment of a human DNA bank in a small and geographically isolated island: The case of Sao Miguel (Azores).** *L. Mota-Vieira<sup>1</sup>, P.R. Pacheco<sup>1</sup>, M.L. Almeida<sup>2</sup>, R. Cabral<sup>1</sup>, J. Carvalho<sup>2</sup>, S. Costa<sup>1</sup>, C. Matos<sup>2</sup>, C.C. Branco<sup>1</sup>, M. Loura<sup>2</sup>, B.R. Peixoto<sup>1</sup>, A.L. Araujo<sup>2</sup>, P. Mendonça<sup>2</sup>.* 1) Genetics & Molec Pathol Unit; 2) Hematology Dept, Hosp Divino Espirito Santo, Azores Islands, Portugal.

Azores is a Portuguese archipelago located in north Atlantic Ocean, corresponding to the western extreme of Europe. Here, we describe the strategy used to build a DNA bank of the population living in the Azorean island of Sao Miguel (131,609 inhabitants) and the analyses of the distribution and geographic origin of the first 350 samples (Dec. 2002 through May 2003). This DNA bank is based on a population sampling of 1,000 healthy unrelated individuals (about 0.8% of the current population), obtained through a collaboration with the Hematology Dept. of the Hospital of Divino Espirito Santo, the only hospital located in S. Miguel island. This DNA bank follows the international ethical guidelines and has been approved by the Hospital's ethical committee. Blood samples (7.5 mL) were collected with appropriate Informed Consent, which includes (1) the distribution of free informative leaflet explaining the goals of proposed studies, the confidentiality of the personal data, and the methods of identification and storage of DNA samples; and (2) an interview with a health professional for additional information. All samples in the repository are anonymous and have self-reported data concerning sex, age, birth and current living places (locality and municipality in the island), and parental birthplaces. To date, we collected 350 blood samples, of which 309 (88%) are male. The mean age of all participants is 36.5y (20-62y). All the six municipalities of the island are represented. The analysis of birthplaces shows that 295 (84%) of all participants have both parents born in S. Miguel island. Moreover, 172 (58%) of these participants have both parents born in the same locality. This data corroborates the high rate of marriages within the rural localities. Thus, the DNA bank of S. Miguel island represents a important resource for population and biomedical genetic research and provides opportunities for us to participate in international collaborative projects. Funded by DRCT, Azores. (lmotavieira@hdes.pt).

**Technology or talk? Equally effective: computer program and an oral presentation in a Tay Sachs disease carrier screening program for secondary school students.** *M.A. Aitken<sup>1,2,3</sup>, A.A. Gason<sup>1,2,3</sup>, S.A. Metcalfe<sup>1,2,3</sup>, E. Sheffield<sup>4</sup>, M.B. Delatycki<sup>5</sup>.* 1) Genetics Education Unit, Murdoch Childrens Res Inst, Parkville, Australia; 2) Dept Paediatrics, The University of Melbourne, Australia; 3) Cooperative Research Centre for the Discovery of Genes for Common Human Diseases, Australia; 4) Genetic Health Services Victoria, Royal Children's Hospital, Parkville, Australia; 5) The Bruce Lefroy Centre for Genetic Health Research, Genetic Health Services Victoria and Murdoch Childrens Research Institute Parkville, Australia.

Tay Sachs disease (TSD) is an inherited neurodegenerative disorder, fatal in children usually by the age of five. Identification of the enzyme deficient in TSD, hexosaminidase A, allowed the implementation of carrier screening programs within the high risk Ashkenazi Jewish community. The Melbourne TSD carrier screening program includes secondary school students and may be used as a model for future screening programs. Therefore, an efficient and flexible educational approach is important. This study compares the effectiveness of a recently developed computer based educational resource with an oral presentation for delivering information to enable informed decision making for testing. Over two years, 348 students completed a pre and post education questionnaire relating to their knowledge, interest, attitudes and anxiety. There was an overall increase in students knowledge following education (1.670.2 points,  $p < 0.0001$ ). The computer program (1.590.21 points) was just as effective for increasing students' knowledge score as the oral presentation (1.600.19 points). Computer based education is widely integrated into school curricula offering a number of advantages such as allowing individuals to be educated at a time that is convenient to them without the necessity of a consistent and experienced educator. Genetic screening programs can use computer based educational approaches as an alternative to face-to-face education without undermining their educational outcomes both in the school setting as well as in the community at large.

**Haemscreen: a Workplace Hemochromatosis Screening Program.** *M.B. Delatycki<sup>1, 2</sup>, A. Nisselle<sup>1, 2</sup>, K.J. Allen<sup>2</sup>, Haemscreen Group.* 1) Bruce Lefroy Centre, MCRI, Royal Children's Hosp, Parkville, Australia; 2) MCRI, Royal Children's Hosp, Parkville, Australia.

Hereditary hemochromatosis (HH) is a common preventable iron overload disease, most often due to homozygosity for the C282Y mutation in HFE. We are piloting a workplace-screening program for C282Y and aim to assess test acceptability and understanding of genetic information for HH.

Employees were invited to an education session and offered screening for C282Y by cheek brush DNA sampling. Questionnaires were administered prior to testing and to C282Y homozygotes and controls four weeks after receiving results. A cohort of non-attenders was surveyed to determine the reasons for non-attendance.

6495 people eligible for testing attended education sessions. 98.0% had testing. The attendance rate was 6.5%. C282Y results are available for 5626 and there have been 22 homozygotes (1:256) and 638 heterozygotes (1:9).

The education resulted in a good understanding of the cause of HH (84% correct) and how it can be prevented (90%) but poorer understanding of penetrance (50%) and genetic and allelic heterogeneity (65%).

Of the 22 homozygotes (age range 18-59), 8 were male. 3/22 were already aware of their genetic status. Of 17 previously unaware whose iron status is known, 6 had markedly abnormal iron studies and have commenced treatment.

The main reason for non-attendance (n= 262) was related to practical constraints in 62% (eg: didnt know about the program), low iron or being a blood donor (9%), HH unimportant or hadnt heard of it (4%), concerns about anxiety related to being found to be C282Y homozygous (1%) and insurance and confidentiality concerns (2%).

In conclusion, of people attending the education session almost all have testing. People understand the cause and prevention of disease but understand the genetic aspects of HH less well. 35% of those unaware of their homozygous state had markedly abnormal iron studies and are undergoing venesection.

**Cooperation in medical genetics in Latin America.** *V.B. Penchaszadeh<sup>1</sup>, A. Giraldo<sup>2</sup>, R. Giugliani<sup>3</sup>, S. Kofman<sup>4</sup>.* 1) Beth Israel Med Ctr, W.H.O. Collab Center for Community Genetics, New York, NY; 2) Genetics Institute, Medical School, National University, Bogota, Colombia; 3) Dept Medical Genetics, Federal University RGS, Porto Alegre, Brazil; 4) Dept Genetics, General Hospital, Mexico City, Mexico.

Latin America is the region of the third world with the longest history and development of human and medical genetics. Current epidemiological transition determines rising proportion of morbidity and mortality due to birth defects and genetic disorders. However, medical genetic services are incipient and fragmented, with coverage reduced to the well off. A meeting of medical genetics experts from Argentina, Brazil, Chile, Colombia, Costa Rica, Ecuador, Mexico, Paraguay and Peru was convened by the World Health Organization in Porto Alegre, Brazil, in June 19, 2003. The group reviewed the status of medical genetics services and research in those countries and designed an agenda for collaboration in specific projects aiming at improving services, education and research for the prevention and management of genetic disorders and birth defects in the region. Task forces were created on specific subjects: clinical genetics services and genetic counseling, genetic testing laboratories, newborn screening, undergraduate and postgraduate education in medical genetics, collaborative research, and ethical, social and legal issues. These groups will work within the recently created Latin American Network of Human Genetics ([www.relagh.ufrgs.br](http://www.relagh.ufrgs.br)) and start concerted actions to further the stated goals. A coordination committee constituted by three of the authors (VBP, AG and RG) will serve as liaison with the different working groups and with non-genetic health professionals, parent/patient organizations, public health officials and legislators. Expected products of this work include prioritization of preventive actions and organization of genetic services according to health status and other factors in each country, model curricula for the teaching of genetics in schools of health sciences, and education modules in genetics for primary health care workers, general public and policymakers.

**Development of a PCR- and minisequencing-based assay for congenital adrenal hyperplasia (21-hydroxylase deficiency).** *D. Keen-Kim, M. Eachus, J. Nakamoto, R. Fenwick.* Dept. Molecular Endocrinology, Quest Diagnostics Nichols Institute, San Juan Capistrano, CA.

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder caused by defects in adrenal steroid biosynthesis, resulting in reduced corticoid and increased androgen production. Approximately 90% of CAH cases are caused by mutations in the 21-hydroxylase gene (*CYP21A2*). Genomic rearrangements between *CYP21A2* and *CYP21A*, a nearby and highly homologous pseudogene, are responsible for the majority of mutations. Both deletions and gene conversion occur. Mutation detection is useful for carrier screening and prenatal diagnosis as well as for neonatal and adult diagnosis in selected cases. Southern hybridization detection of *CYP21* rearrangements, followed by intragenic mutation detection based on nine allele-specific PCR reactions, is typically used. These methods are both laborious and time-consuming. We have designed an allele-specific PCR assay to detect rearrangement at the *CYP21* locus, followed by minisequencing, to detect *CYP21A2* mutations. **Methods:** *CYP21A2*, *CYP21A*, and each of the *CYP21A2/A* and *CYP21A/A2* fusion products produced by genomic rearrangement are amplified using PCR. The nine common intragenic mutations (P30L, In2G, Delta 8bp, I172N, exon 6 cluster, V281L, F306+t, Q318X, R356W) and P453S are subsequently detected by primer extension with fluorescently-labeled dideoxynucleotides and electrophoretic separation on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Data from the ABI 310 are then analyzed with GeneScan and Genotyper software (Applied Biosystems). To validate our method, we assayed, in a blind manner, 13 samples collected from CAH-affected individuals and 10 samples from normal controls. **Results:** The resultant genotypes were 100% concordant (23/23) with the known phenotypes. Turnaround time was 2 days, relative to more than 5 days for Southern hybridization methods. **Conclusions:** We have developed an accurate PCR-based method suitable for CAH (21-hydroxylase deficiency) diagnosis and carrier screening. Moreover, the method has reduced turnaround time compared with other currently used methods.

**Recruitment of Hispanics in Texas and New Mexico into the Cancer Genetics Network using culturally appropriate educational materials.** *L.L. Ballinger<sup>1</sup>, K.J. Gallion<sup>2</sup>, A.R. Miller<sup>4</sup>, G.P. Mineau<sup>3</sup>, A.G. Ramirez<sup>2</sup>.* 1) Cancer Research and Treatment Ctr, Univ. New Mexico, Albuquerque, NM; 2) Chronic Disease Prevention and Control Research Ctr, Baylor College of Medicine, San Antonio, TX; 3) Huntsman Cancer Inst., Univ. Utah, Salt Lake City, UT; 4) Cancer Therapy & Research Ctr, San Antonio, TX.

To increase the participation of Hispanics in cancer genetics research, the Texas Cancer Genetics Network (TCGN) and the Rocky Mountain Cancer Genetics Coalition (RMCGC) are collaborating in a study to test culturally appropriate recruitment materials. This study will evaluate the effectiveness of a magazine, developed by the TCGN, as a recruitment device for the Cancer Genetics Network (CGN) registry. The 24 page, 4-color, English/Spanish, culturally and literacy appropriate magazine is aimed at increasing interest in cancer research. In Texas, Hispanic registrants from tumor registries in San Antonio will be recruited to participate in this CGN recruitment study. This group consists of English and Spanish speaking persons at an increased cancer risk (having had some form of cancer or having family history of cancer). Individuals will be randomly selected into three different conditions: those who receive standard materials via mail; those who receive standard materials plus the magazine; and those who receive standard materials plus the magazine, as well as phone contact. In New Mexico, subjects will be recruited from clinic sources and the general community through advertisements and flyers. Subjects are randomized into receiving standard study recruitment materials with and without the magazine. All subjects receive phone contact if they do not respond to the mailing. The outcome of this study for both sites will be the completion of the standard CGN registry materials and enrollment into the CGN. Participants and those who refuse enrollment will complete questionnaires regarding the effectiveness these recruitment strategies.

Program Nr: 1394 from 2003 ASHG Annual Meeting

**Parental Satisfaction with Active Informed Consent for Expanded Newborn Metabolic Screening.** *S.M. Au<sup>1</sup>, L.E. Hasegawa<sup>2</sup>, C.A. Matsumoto<sup>1</sup>*. 1) Genetics Program, Hawaii Department of Health, Honolulu, HI; 2) Research Corporation of the University of Hawaii, Honolulu, HI.

The first major overhaul of newborn metabolic screening (NBMS) in the past thirty years brings to the forefront the question of whether informed consent should be required before the screening test is performed. Expanded NBMS using tandem mass spectrometry (MS/MS) may detect some disorders which may not have available treatment(s) to date. The State of Hawai'i has been conducting a pilot study examining expanded NBMS with informed consent actively obtained by project assistants trained in providing information about the expanded screening. The project assistants spent approximately 15-20 minutes per new mother providing information and answering questions. Over the past year, 78.0% (5,606/7,186) of the parents opted to have the expanded NBMS after the informed consent process. Parents were asked to complete a satisfaction survey after accepting or declining to participate in the expanded NBMS project. Of the parents who answered the survey question, 98.8% (4,565/4,621) indicated that they understood the information provided. However, 31.2% of the parents still had questions after the project assistants explained the project information. The majority of parents who still had questions (87.9%) also indicated that their additional questions were answered satisfactorily by the project assistants.

**The impact of gene patents on the conduct of research.** *M.R. Henry<sup>1</sup>, J.F. Merz<sup>1</sup>, M.A. Weaver<sup>2</sup>, M.K. Cho<sup>2</sup>.* 1) Center for Bioethics, Univ. of Pennsylvania, Philadelphia, PA; 2) Center for Biomed. Ethics, Stanford Univ., Palo Alto, CA.

**Introduction:** We interviewed researchers who study one of seven specific human genetic conditions to examine if gene patents affect their ability to conduct research. We provide new data on the extent to which patenting and licensing of disease genes is affecting basic research and the development of DNA-based diagnostics and therapeutics. We describe the role of patents in research topic choice, collaboration, competition, material and information sharing, funding, and product commercialization. **Methods:** We conducted semi-structured telephone interviews averaging 35 minutes each with 121 researchers from both non-profit and commercial institutions involved in gene discovery, basic mechanism research, or diagnostic and therapeutic development. Researchers involved in Canavan disease, Chronic Myelogenous Leukemia, Colon Cancers, Cystic Fibrosis, Factor V Leiden, Hereditary Hemochromatosis, or Spinal Muscular Atrophy were identified through patient advocacy websites, the MEDLINE and US Patent and Trademark Office databases, and referral by other study participants. **Results:** Researchers have begun to formalize opinions about the impact of gene patenting on their work. Trends that emerged from our interviews include: academic researchers see patents as a necessary tool to attract industry partners for the development and commercialization of diagnostic tests and therapeutics as well as direct research sponsorship; patents are seen as a reward for discovery rather than a motivation to pursue particular avenues of research; while publication may be delayed due to patent issues, patents are not perceived as placing roadblocks or barriers to research as many researchers believe that non-profit research is exempt from patent infringement; and patents have had little effect on collaborative behaviors. The biggest problem pertaining to patents is that negotiating rights to transfer materials, whether a license or a material transfer agreement, is resource-consuming and may delay or even block research. **Conclusion:** Patents are a secondary thought for the majority of researchers, and neither drive nor substantially hinder research.

**A surveillance of parental informed consent on newborn screening in southern Taiwan.** *C.K. Lee<sup>1</sup>, M.C. Huang<sup>1</sup>, I.C. Lu<sup>2</sup>*. 1) Department of Nursing, National Cheng Kung University, Tainan, Taiwan; 2) Department of Nursing, Chung Hwa College of Medical Technology, Tainan, Taiwan.

**Purpose:** The purpose of this study is to explore the parental consent on newborn screening (NBS) today. **Methods:** Twenty-three obstetric clinics/hospitals executed NBS in southern Taiwan were interviewed. Using content analysis, the transcriptions from interview and relevant document were analyzed to develop the framework of informed consent process in current practices. **Results:** The informed consent processes in current practices of NBS vary widely, and the quality of information provision before obtaining consent is unstable. Most informed consent model is similar to "informed dissent". In few clinics/hospitals, NBS was incorporate into routine pediatric practices. **Conclusions:** The government needs to establish specific legislation to regulate the informed consent process of the new NBS items, and advocates the information about NBS through mass media to educate public. It is crucial to establish the ethical basis of its informed consent model in Taiwan. Further study investigate parental responses to NBS is necessary.

**When genetic research findings progress to clinical application: the ethics of duty to warn.** *D. Pullman<sup>1</sup>, K. Hodgkinson<sup>2</sup>, S. Connors<sup>2</sup>, A. Latus<sup>1</sup>, B. Barrowman<sup>1</sup>, L. Thierfelder<sup>3</sup>, A. Bassett<sup>4</sup>, P. Parfrey<sup>2</sup>, J. Dean<sup>5</sup>.* 1) Medical Ethics, Memorial University, St. John's, NL, Canada; 2) Patient Research Centre, Health Sciences Centre, St. John's, NL, Canada; 3) Max-Delbrück Centre, Berlin, Germany; 4) Clinical Genetic Research, University of Toronto, Ontario; 5) Department of Medical Genetics, Aberdeen, Scotland.

The collection of DNA for linkage studies may provide useful clinical information prior to the determination of a causative mutation, especially for diseases which are difficult to diagnose clinically, have a significant recurrence risk, a high morbidity and mortality, and for which an effective treatment is available. In eleven large families with autosomal dominant arrhythmogenic right ventricular cardiomyopathy (ARVC) in Newfoundland, a founder haplotype defines risk status. ARVC is sex-limited, and severe: 50% of high-risk (HR) males (based on clinical, DNA and pedigree data) are deceased by 40y in the absence of treatment compared with 5% HR females. Implantable cardioverter defibrillator therapy is effective at preventing early sudden death. The use of research haplotype information for clinical management introduces ethical dilemmas between subjects rights and researchers duty of care. Two ARVC subjects with the HR haplotype declined to hear their genetic result. One had several adult offspring, now at 50% risk, the other had employment where sudden death would place many individuals at risk. The ethical principles of beneficence and non-maleficence may imply a duty to warn the at 50% risk adult offspring and to reduce the risk to the public, but this would violate subject confidentiality. The use of anonymous research samples is possible, but this would restrict follow-up research e.g. genotype-phenotype correlations or linkage confirmation. Further, many researchers want to provide predictive genetic information to subjects for serious conditions with effective therapy. We suggest that a desire not to be informed of a possible future genetic result should be an exclusion criterion in research using identifiable samples. In this way, a balance between duty to warn and subject autonomy can be struck.

**Automated Haplotyping in *BRCA1* and Localization of Polymorphisms on Alleles.** *T. Judkins*<sup>1</sup>, *C. Schwensen*<sup>1</sup>, *B.C. Hendrickson*<sup>1</sup>, *H. Harpending*<sup>2</sup>, *J. Barrus*<sup>1</sup>, *T. Scholl*<sup>1</sup>. 1) Myriad Genetic Laboratories, Salt Lake City, UT; 2) Department of Anthropology, University of Utah, Salt Lake City, UT.

Ten canonical haplotypes, defined by 14 common, biallelic polymorphisms detected by DNA sequencing during clinical genetic testing of *BRCA1* describe approximately 99% of chromosomes. An algorithm was developed that defines patient haplotypes from these SNPs using only existing, completely anonymized data. This method successfully assigned haplotypes to 99% of 25,000 individuals and the prevalence of these haplotypes concurred with published data. Previous sequencing had identified 1048 different genetic variants of *BRCA1* in these individuals. Of these, 453 were detected more than once, 411 (over 90%) of which could be assigned to one or more haplotypes.

A phylogenetic tree was created for these *BRCA1* alleles. This tree depicts *BRCA1* bifurcating into two distinct clades. The prevalence of haplotypes within this data set indicates divergence from ancestral alleles in both clades. An analysis of intragenetic variability reveals a paucity of polymorphisms in one of these clades and suggests that it has arisen recently. Conversely, two of the least prevalent alleles, which lie between the two clades, contain disproportionate numbers of genetic variants. Therefore, these oldest (ancestral) alleles appear to be selected against. Correlation with ethnicity data indicated that these haplotypes were 20 times more prevalent in patients that indicated African ancestry.

This model demonstrates accurate and comprehensive assignment of haplotypes in patients undergoing clinical genetic testing for breast/ovarian cancer predisposition. This method increases the clinical utility of patient data by facilitating projects that identify genetic rearrangement mutations and that determine the clinical significance of intronic variants on RNA splicing. This method may also permit highly automated laboratories to achieve these benefits in a number of genetic analyses.

**Extended Mutation Panel for Population-Based CF Carrier Screening.** *G. Tsukerman*<sup>1, 2</sup>, *N. Mosse*<sup>2</sup>, *K. Mosse*<sup>2</sup>. 1) Genetic Screening Program, Reproductive Genetics Institute, Chicago, IL, USA; 2) Institute for Hereditary Diseases, Minsk, Belarus.

More than 1,000 CFTR alleles have been identified to date. The frequency of most CFTR mutations is highly variable and often depends on the ethnic origin of the patients. In October 2001, ACMG/ACOG jointly recommended CF carrier screening using a panel of 25 mutations. The panel of selected mutations was primarily based on mutation frequency in the Northern European and Ashkenazi Jewish population. In 2000, a large genomic deletion, spanning introns 1-3 of the CFTR gene, was detected in CF patients from Central and Eastern Europe. The mutation, termed CFTRdele2,3(21kb), was particularly common, 1.1-6.4%, in Czech, Russian, Austrian, German, Polish, Ukrainian, Slovenian and Slovak CF patients. It was suggested that the CFTRdele2,3(21kb) deletion could have originated in a common Slavic ancestral population (Dork T. et. al., 2000). Analysis of 244 CF alleles from Belarus patients has shown that the delta F-508 mutation covered 63% of CF chromosomes, R334W 0.8%, R347P 0.4%, G542X 1.3%, S549N 0.4%, R553X 0.4%, W1282X 0.9%, N1303K 3.7%, 2184delA 3.7%. The CFTRdele2,3(21kb) deletion accounted for 6.7% of all CF chromosomes and became the second most common CF mutation in Belarus after delta F-508. This observation can be additional evidence of Slavic ancestry of that deletion. Currently, in the USA, (ACMG 25, Quest 32, Genzyme Genetics 87) no CF mutation panel includes the CFTRdele2,3(21kb) deletion. Taking into consideration the fact (US Census Bureau, 2000) that more than 10 million US residents have Slavic Central and Eastern European origin it seems justified to add the CFTRdele2,3(21kb) deletion to the mutation panel for CF carrier screening. CF carrier screening for the CFTRdele2,3(21kb) deletion should substantially improve the detection rate of the mutations in European American couples of Slavic descent.

**Analysis of dystrophin mRNA show nonsense, splice and cryptic splice site mutations cause Becker muscular dystrophy.** *S.C. Yau, Z. Hatton, V. Nihalani, R.G. Roberts, S.J. Abbs.* Genetics Centre, Guy's Hospital, London, UK.

We have applied the protein truncation test to screen muscle RNA from 9 BMD patients with no gross rearrangements of the dystrophin gene. Four cryptic splice site mutations were identified. An A to G change 2 kb into intron 25 of BMD1 creates a cryptic acceptor site, resulting in two mutant cDNAs with 95bp and 207bp of intronic sequence inserted between exons 25 and 26. The effect on RNA splicing is similar to the donor site mutation reported by Ikezawa et al., despite the mutations being 206bp apart. This same acceptor site mutation has also been reported by Tuffery-Giraud et al. but only results in the 95bp insertion.

In BMD2 an A to T change 636 bp 3 of exon 32 creates a cryptic acceptor site adding 43bp of intronic sequence between exons 32 and 33. An A to T change 9kb 5 of exon 45 creates a cryptic donor site in BMD3 and results in an extra 71bp incorporated between exons 44 and 45 in the cDNA. In BMD4 an A insertion more than 55kb 5 of exon 45 produces a cryptic donor site causing an extra 74bp insertion in the cDNA. In all cases despite premature stop codons, alternative splicing produces full-length protein, accounting for the milder phenotypes.

Nonsense mutations in exons 25 and 49 (BMD5 & 6) and donor site mutations in exons 4 and 64 (BMD7 & 8) were found to cause alternative splicing of inframe exons in the mRNA. The 1bp deletion at the donor site of exon 64 also resulted in an additional methionine residue in BMD8s dystrophin. Although BMD9 has an out-of-frame 5bp deletion in exon 71, it is thought that failure of nonsense mediated decay means that some mutant mRNA is not completely degraded but is translated into near full-length dystrophin that can rescue the predicted severe phenotype.

Four of these mutations would not have been detectable with DNA-based mutation screening strategies. In order to offer comprehensive mutation screening in genes with large introns such as dystrophin, an RNA-based approach is likely to be required for the foreseeable future.

**Diagnostic testing for Cystic Fibrosis by MALDI-TOF mass spectrometry: Identification of a CF patient with a G542X/3199del6 genotype.** *I.M. Buysse<sup>1</sup>, S.E. McCarthy<sup>1</sup>, R.P. Pace<sup>1</sup>, E.S. Schmitt<sup>1</sup>, P. Ward<sup>1</sup>, C. Oermann<sup>2</sup>, C.M. Eng<sup>1</sup>, B.B. Roa<sup>1</sup>.* 1) Medical Genetics Laboratory, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pediatrics, Baylor College of Medicine, Houston, TX.

Cystic fibrosis diagnostic testing is currently performed in our laboratory using the MALDI-TOF mass spectrometry platform (Sequenom). This PCR-based assay employs oligonucleotide primer-extension, followed by discrimination of specific extension products by mass spectrometry. This high-throughput system was successfully validated against our previous allele-specific oligonucleotide (ASO) hybridization assay by parallel testing on >1000 patient samples. Since implementation, we have tested >2000 patient samples using the expanded MALDI-TOF assay. Our current CF panel contains the 25 core mutations recommended by ACMG, plus additional CF alleles including the 3199del6 allele. This was previously reported as a potential modifier for the I148T allele, wherein classic CF patients have a complex I148T/3199del6/9T genotype on one chromosome, paired with a CF mutation on the other chromosome (1). The clinical significance of the 3199del6 allele without I148T is unclear given the current limited data. In the course of our CF clinical testing, we studied a 2 year old Hispanic male with an atypical CF presentation consisting of meconium ileus, positive sweat test, malabsorption that resolved following pancreatic enzyme supplementation, and absence of respiratory symptoms at this time. He tested positive for the G542X mutation and the 3199del6 allele, but negative for I148T; he was compound heterozygous for the 7T/9T alleles. Additional studies are being pursued, including parental analysis to determine haplotypes, and *CFTR* gene sequence analysis to assess for a possibly unidentified mutation. These studies could help elucidate the clinical significance of the 3199del6 and I148T alleles, and could potentially illustrate the value of including the 3199del6 allele in routine CF testing.

1. Rohlfs et al. *Genet. Med.* (2002), 4: 319-323.

**Genotype-phenotype correlation between the complex allele I148T-3199del6 and Cystic Fibrosis.** *A.F. Ruchon, S.R. Ryan, R. Rozen, P. Scott.* Molecular Genetics Laboratory, McGill University Health Center, Montreal, Quebec, Canada.

Over the past decade, mutation analysis of the CFTR gene in Cystic Fibrosis patients has identified more than 1000 mutations. Only a small number of these mutations result in a predictable disease phenotype and are classified as severe or mild, based on their effect on protein function and/or the resulting phenotype. The I148T mutation is considered a severe mutation and is included in the ACMG/ACOG recommended CF mutation screening panel. Recently, it has been suggested that I148T by itself is not responsible for the severe phenotype. Rather the severity appears to be due to an association in cis of I148T and 3199del6. The 3199del6 mutation is an in-frame deletion in exon 17 that removes two conserved amino acids from the second membrane-spanning domain of the CFTR protein, likely resulting in the absence of functional protein. We have studied 24 CF patients, originally identified as carrying the I148T mutation and a second severe CFTR mutation, for the presence of 3199del6. All our patients have the deletion and are of French Canadian descent. Genotype-phenotype correlation for this complex allele has been established based on the characterization of the clinical status at the time of diagnosis and thereafter. To date, available clinical information confirms the previous observation of a classical CF phenotype based on pancreatic status, sweat test and pulmonary function associated with this complex allele.

Program Nr: 1403 from 2003 ASHG Annual Meeting

**Extensive survey of *ABCA4* mutations with a commercial gene chip in a cohort of patients with Stargardt Disease or retinitis pigmentosa.** *A.N. Yatsenko*<sup>1</sup>, *R.A. Lewis*<sup>1,2,3,4</sup>, *W. Wiszniewski*<sup>1</sup>, *J.R. Lupski*<sup>1,2</sup>. 1) Molecular & Human Genetics; 2) Pediatrics; 3) Medicine; 4) Ophthalmology, Baylor College of Medicine, Houston, TX.

Mutations of *ABCA4* (*ABCR*) have been associated with several retinal disorders including Stargardt Disease (STGD) and recessive retinitis pigmentosa (arRP). However, the large size of the gene and heterogeneity of *ABCA4* variants (to date ~400 alteration variants) have confounded genetic analyses and diagnoses. To resolve these problems, a fast and affordable test, an *ABCA4* genotyping microarray screening of known *ABCA4* alterations, has been developed recently by ASPER (Tartu, Estonia). The test uses an ABCR-400 DNA chip created with allele-specific primer extension (APEX) technology. To validate efficiency and to estimate mutation detection rates for this novel technology, we screened 162 independent STGD, 30 arRP, and control samples with known *ABCA4* mutations. We confirmed the presence of identified mutations by both sequence and segregation analyses. Our preliminary data indicate that this microarray technology identifies reported disease-associated *ABCA4* alleles with ~98-99% accuracy. We estimate a mutation detection rate near 60%. This is perhaps the first extensive study of *ABCA4* mutations in patients with recessive RP. Additional studies with an ethnically diverse cohort of STGD patients will validate this technology. We suggest that microarray screening with subsequent sequence analysis confirmation will be sufficient to confirm the clinical diagnosis of classic Stargardt disease and other *ABCA4*-associated retinopathies.

**Novel contributions to the Asian CFTR mutation spectrum: genotype and phenotype in Thai patients with cystic fibrosis.** *S. Ramalingam*<sup>1</sup>, *R. Sankaran*<sup>1</sup>, *W. Karnsakul*<sup>2</sup>, *P. Gardner*<sup>1</sup>, *I. Schrijver*<sup>3</sup>. 1) Medicine, Stanford University Medical Center, Stanford, CA, USA; 2) Pediatrics, Siriraj Hospital, Mahidol University, Bangkok, Thailand; 3) Pathology, Stanford University Medical Center, Stanford, CA, USA.

Cystic fibrosis (CF) is an autosomal recessive disorder caused by CFTR mutations. Due to the prevalent F508 mutation, CF is common in Caucasians and the mutation spectrum in this group is well characterized. Other ethnic groups, however, have not been analyzed in depth. As yet, only one mutation has been reported from Thailand. We studied three Thai CF patients, who presented with hyponatremic hypochloremic metabolic alkalosis in the first months of life. The CFTR coding region and intervening sequences flanking the 27 exons were amplified by PCR, followed by direct DNA sequencing. Five mutations were identified, three of which are novel. Patient 1, a child of parents with Chinese ancestry, is thus far free of lung disease and pancreatic insufficiency. One of his mutations (4330delTG in exon 23) is associated with a mild phenotype in another patient. The second mutation (3206 C>A in exon 17a) leads to A1025D and has not yet been described. Patient 2 has classic CF. He carries a splice site mutation in IVS 3 (405+1G>A) and the novel 1001+3A>T in IVS 6b. This mutation is expected to also result in alternative splicing and mRNA studies will be performed. Patient 3, a child of non-consanguineous parents, presented with pancreatic insufficiency and pneumonia at four months of age and expired of respiratory failure and sepsis. One of her siblings had an identical clinical course. DNA analysis revealed homozygosity for a novel nonsense mutation in exon 6a (738G>A, W202X). A larger study is ongoing to determine prevalence of these mutations in the Thai population. Our results support the notion that CFTR mutations in non-Caucasians are under-studied, and that Asian individuals may carry specific mutations that remain undetected by limited mutation panels. Further analysis of this mutation spectrum will allow population-specific genetic analysis in Thailand, and improve diagnostic sensitivity in the multi-ethnic US population.

**Newborn screening for  $\alpha$ -thalassemia: molecular genetic confirmatory testing by multiplex-ARMS.** *U. Bhardwaj*<sup>1</sup>, *Y.-H. Zhang*<sup>1</sup>, *F. Lorey*<sup>2</sup>, *L.L. McCabe*<sup>1,3</sup>, *E.R.B. McCabe*<sup>1,3,4</sup>. 1) Dept Pediatrics, David Geffen Sch of Med at UCLA, LA, CA; 2) Genetic Disease Branch, CA Dept of Health Services, Berkeley, CA; 3) Dept of Human Genetics, David Geffen Sch of Med at UCLA, LA, CA; 4) Mattel Children's Hosp at UCLA, LA, CA.

$\alpha$ -Thalassemia ( $\alpha$ -thal) is an increasing health problem in the US due to changing demography. Many US neonatal screening laboratories use high performance liquid chromatography or isoelectric focusing, which may result in diagnostic confusion due to interaction of various hemoglobinopathies with  $\alpha$ -thal. We developed single-tube multiplexed PCR assays using original neonatal screening specimens to identify the mutations responsible for  $\alpha$ -thal to expedite diagnostic confirmation. Primers were designed for 2-6 common ethnic-specific mutations using the amplification refractory mutation system (ARMS) and were standardized on positive control samples. The multiplex-ARMS approach was validated using DNA samples with known  $\alpha$ -thal mutations. Specimens from African-American neonates were tested for two mutations (-88 and -29); Asian Indians for five mutations (IVSI-1, IVSI-5, Cd 41/42, Cd 8/9 and 619 bp del); Chinese for five mutations (Cd 41/42, Cd 17, -28, Cd 71/72 and IVSII-654); and Southeast Asians for six mutations (Cd 41/42, Cd17, -28, IVSII-654, IVSI-5 and IVSI-1). We identified  $\alpha$ -thal mutations by multiplex-ARMS from positive control samples, confirming successful development and application of the assay. We tested 25 anonymized dried blood specimens from neonates belonging to these ethnic groups who had  $\alpha$ -thal. We detected a mutation using the ARMS approach in nearly all specimens: four Asian Indian mutations in five samples; two Chinese mutations in seven samples; two Southeast Asian mutations in 12 samples; and one African-American mutations in one sample. Results were confirmed by sequencing. Multiplex-ARMS for ethnic-specific  $\alpha$ -thal mutations using original newborn screening dried blood specimens is a rapid and efficient approach for diagnostic confirmation. Implementation of DNA confirmatory testing for  $\alpha$ -thal will not only improve early diagnosis, but also will facilitate genetic counseling in these families.

**Frequency of the single deletional alpha-thalassemia allele ( $-^{3.7}$ ) in the different racial groups in Malaysia - A molecular study.** *Y.C. Wee<sup>1</sup>, J.A.M.A. Tan<sup>1</sup>, K.L. Tan<sup>1</sup>, W.P. Chow<sup>2</sup>, S.F. Yap<sup>3</sup>, E. George<sup>4</sup>.* 1) Department of Molecular Medicine, University of Malaya, Kuala Lumpur, Malaysia; 2) Department of Obstetrics and Gynecology, University of Malaya, Kuala Lumpur, Malaysia; 3) Department of Pathology, University of Malaya, Kuala Lumpur, Malaysia; 4) Department of Clinical Laboratory Sciences, Faculty of Medicine and Health Sciences, University Putra Malaysia, Selangor, Malaysia.

Alpha-thalassemia is one of the most common genetic Hb disorders in Malaysia and it is a public health problem. The disorder can result from deletional or non-deletional (mutations) of the  $\alpha$ -thalassemia genes. In Malaysia,  $\alpha$ -thalassemia is most often caused by gene deletions resulting in  $\alpha$ -thalassemia 1 ( $^0$ ) and  $\alpha$ -thalassemia 2 ( $^+$ ). Individuals with  $^0$ -thalassemia ( $--/$ ) possess a molecular defect where both  $\alpha$ -globin genes in cis are deleted, and this defect has been reported to be more commonly present in the Malaysian Chinese. Individuals with  $^+$ -thalassemia have one  $\alpha$ -gene deleted ( $-/$ ) and this was reported to be more common in the Malays. In this study of  $^+$ -thalassemia, the frequency of the single deletional alpha-thalassemia allele ( $-^{3.7}$ ) was characterized in 670 blood samples: 402 from the Malays, 148 from Indians, 100 from Chinese and 20 from other ethnic groups. The defect was studied using DNA amplification to detect the  $-^{3.7}$  rightward deletion and the normal 2-gene sequence in two separate reactions. Specific primers were used to amplify the 2 and 1 genes. One reaction amplifies the 2-1 genes flanking the  $-^{3.7}$  deletion region to detect the presence of  $-^{3.7}$  rightward deletion while the second reaction amplifies a comparable segment of the normal 2 gene. Both DNA amplification protocols produce a molecular weight product of 1.8 kb. The  $-^{3.7}$  deletional defect was confirmed in 11 % of Malays, 10 % of Chinese and 6.1 % of the Indians studied. The defect was also detected in a patient with Thai nationality and in an Orang Asli (Aborigine) patient belonging to the Bidayuh tribe. In conclusion, the  $^+$ -thalassemia ( $-/$ ) defect was found in similar frequencies in the Malays and Chinese and at a lower frequency in the Indians in Malaysia.

**Extensive Sequencing of the  $\beta$ -Globin Gene for the Detection of  $\beta$ -thalassemia Mutations.** *F. Quan<sup>1</sup>, S. Wang<sup>1</sup>, C.M. Strom<sup>1</sup>, M.V. Gallican<sup>2</sup>, W. Sun<sup>1</sup>.* 1) Molecular Genetics, Quest Diagnostics Nichols Institute, San Juan Capistrano, CA; 2) Hematology, Quest Diagnostics Nichols Institute, Chantilly, VA.

$\beta$ -thalassemia is characterized by the reduced or absent synthesis of  $\beta$ -globin chains. Most common causes of the  $\beta$ -thalassemia are point mutations in the  $\beta$ -globin gene. To date, over 200  $\beta$ -globin mutations have been reported. The individuals homozygous for a  $\beta$ -thalassemia mutation, or compound heterozygous for 2 different mutations, may manifest with  $\beta$ -thalassemia major or intermedia that is characterized by severe anemia, skeletal deformities, and hepatosplenomegaly. Carriers of one  $\beta$ -thalassemia allele can be normal, or have mild anemia. Traditional hematological tests (red blood cell indices, peripheral blood smears, and hemoglobin analysis) can diagnose patients with typical  $\beta$ -thalassemia. However, it remains a challenge for these hematological tests to accurately detect all carriers or make prenatal diagnosis. Here we report the development and validation of a comprehensive  $\beta$ -globin sequencing assay for the detection of  $\beta$ -thalassemia mutations. In this assay, the three exons of the  $\beta$ -globin gene, including the promoter region and their neighboring intronic regions, and part of intron 2 are independently amplified by polymerase chain reaction (PCR) and sequenced in both directions by dye-terminator cycle sequencing. This extensive  $\beta$ -globin sequencing assay can detect all reported point mutations in  $\beta$ -globin gene. This assay has been validated with a panel of 10 DNA samples, including 4  $\beta$ -thalassemia patients with known mutations (total of 7 distinct mutant alleles). All DNA samples were genotyped correctly. We further analyzed DNA samples from 23 patients diagnosed or suspected, by hemoglobin analysis, as carrying  $\beta$ -globin mutation(s). A total of 27  $\beta$ -globin mutant alleles (15 distinct mutations) were detected in 19 of these patients. These studies demonstrated the validity, and clinical utility of the comprehensive  $\beta$ -globin sequencing assay we developed.

**Genetic Basis of Korean Patients with Prader-Willi Syndrome.** *H.J. Jo, C.S. Ki, J.W. Kim.* Dept Laboratory Medicine, Samsung Medical Ctr, Seoul, Korea.

Prader-Willi syndrome (PWS) is clinically distinct complex disorder mapped to chromosome 15q11-q13. PWS can result from microdeletion, uniparental disomy (UPD), or imprinting center defect (ID) in 15q11-q13. Although PWS is not uncommon in Korean population, there is no data on the frequency of each genetic mechanism of Korean patients with PWS. Therefore, we tried to analyze the genetic basis of Korean PWS. A total of 88 cases were analyzed. Chromosome analysis, interphase fluorescent in situ hybridization (FISH) with *SNRPN* probe, and methylation sensitive polymerase chain reaction (m-PCR) were performed with peripheral blood specimens. Clinical findings were scored according to the consensus diagnostic criteria for PWS. Sixteen cases tested with both FISH and m-PCR were confirmed as having PWS either by a deletion of PWS critical region (n=10) or by an abnormal methylation pattern (n=6). Among sixty-seven cases tested with FISH but not with the m-PCR, 15 cases were consistent with a deletion pattern. In addition, five m-PCR positive cases lack FISH data. Taken together, 6 out of 31 (19.4%) confirmed PWS patients showed a PWS-specific m-PCR pattern with normal FISH signal, which could be due to either UPD or ID mechanism. The above results suggest that about 80.6% of Korean patients with PWS may be due to microdeletion of PWS critical region in 15q11-q13. Considering these results, the frequency of UPD or ID seems to be a little lower than those of previously reported data in other populations (~30%). However, if we take into account of 57 cases in which either FISH or m-PCR was done, the proportion of UPD or ID might be greater than it is.

**Ethnicity-specific differences in second-trimester maternal serum screening.** *F. Alvarez Nava, M. Soto-Quintana, T. Padrón, A. Morales-Machín, M.C. Martínez, A. Rojas-Atencio, K. Urdaneta.* Unidad de Genética Médica, Universidad del Zulia, Maracaibo, Venezuela.

Background: Second-trimester maternal serum screening is associated with fetal and perinatal disorders. Effective and accurate screening is based on the establishment of the population-derived median values of AFP, -hCG, and uE<sub>3</sub> in pregnant women. Race-ethnicity-specific differences have been observed. Objective: To identify any race-ethnicity-specific variations between Venezuelan pregnant women and other populations in second-trimester maternal serum screening. Study Design: Maternal serum levels AFP, -hCG and uE<sub>3</sub> were measured in 2,000 unaffected singleton, nondiabetic pregnancies from 14 to 21 weeks in our laboratory, Maracaibo, Venezuela. Patients with chromosome abnormalities or fetal anomalies were excluded. All results were expressed as multiple of the median (MoM) gestation specific values. Data from many studies in Hispanic (born in USA), Caucasian and Afroamerican women were compared with our data. Results: There were variations across gestational ages between different groups. All three analytes in our women appeared to be higher than data from Caucasian women but they are lower than data from Afroamerican women. Differences were seen even when data were compared with Hispanics born in USA. These patterns are considered statistically significant when are compared at advanced gestational ages (p 0.05). Weight correction did not compensate for ethnic variations. Conclusions: Adjustment of medians for Hispanic ethnicity may have a small but significant effect, especially with regard to low values and at advanced gestational age. Use of separate data bases should result in more accurate screening.

Program Nr: 1410 from 2003 ASHG Annual Meeting

**Quality assurance of markers in integrated prenatal screening for Down syndrome.** *C. Meier, T. Huang, A.M. Summers.* Dept Genetics, North York General Hosp, Toronto, ON, Canada.

Maternal serum screening (MSS) for Down syndrome has been offered in the province of Ontario as a funded service since 1993. Quality assurance (QA) of MSS relies on both internal and external analyses of the biochemical markers used (AFP, uE3 and hCG) including periodic review of median MoM values, weight correction and positive rates. In 1999, our laboratory was the first in Canada to offer integrated prenatal screening (IPS), which incorporates the results of first trimester ultrasound (NT) and biochemical (PAPP-A) markers with the standard MSS markers to provide a single, more accurate, risk estimate. We have reported over 16,000 IPS results since introduction and integrated screening currently makes up 29% of the total provincial volume. We have found the methodology of using and monitoring the MoM values of integrated screening markers is effective in assuring the quality of screening results. While QA of biochemical markers is relatively straightforward, the ultrasound marker, nuchal translucency (NT), demands specialized methods to ensure that performance is acceptable. The NT measurements received from 133 sonographers enrolled to date in our screening program are subject to initial assessment and ongoing review. Using sonographer-specific NT medians provides the comparable improvement of risk estimates as does the use of laboratory-specific biochemistry medians, namely, the correction of variance attributed to the instrument and operator. Given that our actual screening performance, with respect to false-positive rates and detection rates, closely approximates the theoretical performance predicted by published sources, we can confirm that MoM-based quality assurance of integrated screening markers is effective.

**Weight Correction and Ethnic Differences for First Trimester Down Syndrome Biochemical Markers.** *D. Krantz, T. Hallahan, J. Macri.* NTD Labs, Huntington Stat, NY.

First Trimester Down syndrome screening using Free Beta hCG, PAPP-A and nuchal translucency is steadily replacing second trimester screening in routine prenatal care. As part of second trimester multiple marker screening weight and ethnic variation have been observed for all of the screening markers. We conducted a study to determine the effect of these factors on first trimester biochemistry. We reviewed the results of 36,635 patients, including, 1872 African Americans, 576 As. Indians, 1412 Asians, 1881 Hispanics, 68 Native Am, 30263 Caucasian and 563 Others. Maternal weight correction factors for free Beta hCG and PAPP-A were developed based on the white ethnic groups. Median MoM results were then compared before and after adjustment. The maternal weight correction formulas were:  $\ln(\text{FB hCG}) = \text{EXP}(3.7087 - .7449 * \ln(\text{wt}))$ ,  $110 \leq \text{wt} \leq 225$  lbs and  $\ln(\text{PA}) = \text{EXP}(6.7495 - 1.3556 * \ln(\text{wt}))$ ,  $115 \leq \text{wt} \leq 275$  lbs  
The Median MoMs for each ethnic group are shown below:

	Afr. Amer	As. Indian	Asian	Hispanic	Native Am	Caucasian	Other
Unadj FB	1.03	1.06	1.15	0.89	0.88	1.00	1.05
Wt.Adj. FB	1.15*	1.00	1.06*	0.89*	0.95	1.01	1.03
Unadj PA	1.09	1.12	1.19	0.98	1.01	1.00	1.01
Wt.Adj. PA	1.30*	0.99	1.03	0.98	1.01	1.01	1.01

\* Indicates significant difference vs. Caucasian group using Bonferroni criteria (overall  $P < 0.05$ ). As with second trimester screening, first trimester Down syndrome biochemical screening markers should be adjusted for maternal weight and ethnicity.

**Quality of human DNA from buccal cytobrushes depends on storage conditions.** P.A. Krakowiak, Y. Guan, J.J. Greenhaw, W.A. Freeman, J. Kimbrough, R. Lyle, W. Zhao, M.A. Cleves, C.A. Hobbs. Pediatrics, UAMS, Little Rock, AR.

**Background:** Obtaining DNA from participants enrolled in large epidemiologic studies is critical to unraveling genetic causes and susceptibilities to complex diseases. Buccal epithelial cell collection is a quick, safe, inexpensive, and noninvasive process of obtaining DNA that can be self-administered and returned via mail. However, the obtained DNA has two major problems: low concentration and bacterial contamination, that can lead to degradation of the human DNA. Our data from 1119 mailed buccal samples indicate that genotyping success rates are significantly better (86%) when cytobrushes are transported during colder months (average monthly high < 68F), compared to months when temperatures are above 68F (57%,  $p < 0.001$ ). Although bacterial contamination has been recognized, the storage conditions that may affect the extent of contamination have not been studied systematically. **Methods:** Using volunteer samples, we conducted experiments to determine the effects of storage time, humidity, temperature, and duration of sample collection on DNA quality and quantity. We calculated microsatellite genotyping success rate, specific gene (*NKX2.5*) PCR amplification success, and DNA quantity using real-time PCR. **Results:** Increased temperature and humidity were the most important elements that reduced the quality of DNA. Genotyping success rates were significantly lower for cytobrushes stored at 37C in air-tight (*humid*) compared to open-to-air (*arid*) containers (48 vs 88%,  $p = 0.003$ ) and when *humid* samples were stored for 5 days vs 1 day (60 vs 85%,  $p = 0.01$ ). *NKX2.5* PCR success scores were also lower for humid vs arid samples (0.45 vs 1.38,  $p < 0.001$ ). When cytobrushes were processed immediately, success rates were not different between a volunteers 1st and 10th brush ( $p < 0.001$ ), and for brushes collected 1 and 2 weeks later ( $p < 0.001$ ). **Conclusion:** When it is impractical to collect DNA by other means, buccal cell samples are a useful alternative. However, storage conditions of cytobrushes may significantly alter the quality of obtained DNA; therefore, they should be transported in containers permeable to air or in a buffer containing a lysis reagent to diminish bacterial growth.

**A new quantitative test to simultaneously screen for galactose uridylyltransferase and biotinidase deficiency in newborns using a single blood spot.** *S.J. Mihalik, J.G. Suzow, D.E. Freer, E.W. Naylor.* Neo Gen Screening Inc., Bridgeville, PA.

Newborn screening relies on the use of small quantities of dried whole blood on filter paper for testing. The large quantities of hemoglobin in whole blood frequently quench both colorimetric and fluorometric assays, and as such its role must be considered in assay design. The purpose of this study was to develop a routine newborn screening protocol in which one eluted blood spot could be used to screen for at least two disorders that are commonly included in newborn screening programs. Uridylyltransferase (classical galactosemia) and biotinidase assays meet the criteria and both utilize enzyme assays. Blood spots (3.2mm) were eluted in water and aliquoted to individual plates using a Packard Multiprobe. Both enzymes were then assayed using modifications of standard protocols, except that the interference from hemoglobin was addressed. For the uridylyltransferase, samples were diluted after the reaction, resulting in the avoidance of quenching of the NADH fluorescence. In the colorimetric biotinidase assay using the biotinyl-*p*-aminobenzoic acid substrate, proteins were precipitated with trichloroacetic acid and the interfering precipitate was removed by filtration before colorimetric quantitation. The assays were found to be both precise and accurate and were found to have a coefficient of variation that was comparable to that of the assays performed using an established continuous flow method. Future plans include the extension of this concept of multiple assays from one extraction to include at least one other enzyme assay. This miniaturization approach to assays continues to ensure that newborn screening is affordable and minimally invasive.

**Molecular Diagnosis of Beckwith-Wiedemann Syndrome: Quantitative Analysis of Methylation in the KvDMR**

**Region.** *B. Baskin*<sup>1</sup>, *R. Weksberg*<sup>2, 3</sup>, *P.N. Ray*<sup>1, 3</sup>, *T.L. Stockley*<sup>1, 4</sup>. 1) Molecular Genetics Laboratory, Department of Paediatric Laboratory Medicine; 2) Department of Clinical and Medical Genetics, The Hospital for Sick Children; 3) Department of Molecular and Medical Genetics; 4) Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada.

Beckwith-Wiedemann syndrome (BWS) is a genetic overgrowth syndrome characterized by a number of features such as somatic overgrowth, abdominal wall defects, macroglossia and embryonal tumours. BWS is associated with genetic or epigenetic abnormalities of several imprinted genes on human chromosome 11p15. These genes usually show differential DNA methylation. A differentially methylated region (DMR) is a DNA sequence that bears a CpG-methylation mark that is specific to the chromosomal parent-of-origin. Such DMRs may be maternally or paternally methylated. One important DMR in BWS is KvDMR1, which is located within intron 10 of the potassium channel coding gene *KCNQ1*. The paternal allele is non-methylated, permitting the paternal expression of a transcript called *KCNQ1OT1* whereas the maternal allele is methylated, keeping the maternal expression of *KCNQ1OT1* silenced. For diagnosis we currently provide UPD analysis which detects mutations in ~15% of BWS patients. The majority of BWS patients, ~60%, show loss of methylation at a certain degree in KvDMR1 on the maternal allele. However there is currently no clinical quantitative molecular test to analyze the methylation status in this region. We are developing a new diagnostic assay, which detects the methylation status in KvDMR1. The technology we are using is Pyrosequencing, a real-time mini-sequencing method, to quantify the amounts of incorporated C/Ts and thereby determine the methylation status at certain specific CpGs in KvDMR1. Using this technology we have been able to develop a new quantitative assay to identify maternal loss of methylation at CpG sites in BWS patients. This test will be validated in the Molecular Genetics Laboratory for diagnosis of BWS patients, as well as for potential use for other DMRs.

Program Nr: 1415 from 2003 ASHG Annual Meeting

**Comprehensive screening of the dystrophin gene.** *T.W. Prior, J.W. Heinz, J. Fang.* Pathology, Ohio State University, Columbus, OH.

Approximately 2/3 of the DMD/BMD population have been shown to have large deletions of the dystrophin gene. We have performed an extensive screen on our nondeletion patients for duplications by Southern blotting the entire gene and for point mutations by DHPLC and sequencing. Unlike the deletion distribution, duplications were found in only about 5-8% of the patients and were located primarily at the 5' end of the gene. 82 nondeletion/nonduplication patients were screened for point mutations by DHPLC and sequencing. Several novel point mutations were identified and were randomly distributed throughout the gene. Nonsense, frameshift and splice mutations all resulting in protein truncation were found. Analysis of intron 1 revealed a splice site mutation in an X-linked dilated cardiomyopathy patient. Several base changes causing significant amino acid substitutions were found, however, these were most likely polymorphic changes. Missense mutations in the dystrophin gene are rare events. The identification of duplications and point mutations is important for the confirmation of the diagnosis and for accurate carrier studies. In the future, molecular therapies (such as antisense oligonucleotides, antibiotics or chimeric RNA/DNA) will be applied according to the specific dystrophin mutation. This will require a complete mutation analysis and identification of all types of dystrophin mutations. (This research has been generously supported by the Muscular Dystrophy Association).

**A Blood Based Diagnostic Test for LGMD 2B and Miyoshi Myopathy.** *A. Hennigan<sup>1</sup>, L. Hayes<sup>1</sup>, M. Tatis<sup>1</sup>, J. Sisson<sup>1</sup>, K. Greenough<sup>1</sup>, L. Kertiles<sup>1</sup>, E. Gallardo<sup>2</sup>, I. Illa<sup>2</sup>, R.H. Brown, Jr<sup>3</sup>, S.D. Batish<sup>1</sup>, W.K. Seltzer<sup>1</sup>.* 1) Athena Diagnostics, Inc., Worcester, MA 01605; 2) Department of Neurology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; 3) Department of Neurology, Massachusetts General Hospital, Charlestown, MA 02129.

Limb Girdle Muscular Dystrophy 2B (LGMD 2B) and Miyoshi Myopathy (MM) are allelic autosomal recessive disorders that arise from defects in the dysferlin (DYSF) gene. The disease, which begins in young adults, is progressive with muscle degeneration resulting from dysferlin protein deficiency. In LGMD 2B, weakness starts in the proximal muscles of the pelvic girdle, while in MM, weakness affects the gastrocnemius muscles and subsequently the proximal muscles. It is estimated that loss of dysferlin accounts for 5% to 10% of all incidences of muscular dystrophy. Associated phenotypes include HyperCKemia and distal anterior phenotype. We describe the development of a diagnostic test for LGMD 2B/MM based on the method of Ho et. al. that screens for dysferlin expression in whole blood. Dysferlin-expressing monocytes are purified, extracts prepared and specific protein detected in Western blotting by using a commercially available monoclonal antibody. Monocyte purification coupled with the use of highly sensitive immunoblotting technique ensures detection of dysferlin protein, if present. This is a critical feature of our test since, by definition, individuals with LGMD 2B/MM have no detectable dysferlin protein. Data obtained during test development demonstrates dysferlin to be a stable protein, detectable in whole blood of normal control individuals up to two days after blood draw. The primary benefit of this technology is the ability it gives clinicians to confirm dysferlinopathy by using a simple blood test. The need to perform a muscle biopsy is therefore eliminated. This poster will present an overview of the testing service and its value in the diagnosis of individuals affected with LGMD 2B/MM.

**Mutational Analysis of ATM Gene in Iranian Patients with Ataxia Telangiectasia.** *H. Atashi Shirazi*<sup>1</sup>, *B. Bayat*<sup>2</sup>, *S. Ahmad. Aleyasin*<sup>2</sup>, *A. Farhoodi*<sup>3</sup>, *M. Moin*<sup>3</sup>, *M. Hosse Sanati*<sup>3</sup>. 1) Tehran Khatam University Tehran, Iran; 2) National Research Center for Genetic Engineering and Biotechnology (NRCGEB), Tehran, Iran; 3) Children Medical Center, Tehran, Iran.

Ataxia telangiectasia is an autosomal recessive disease. More than 100 mutations in ATM gene have been reported which cause ataxia telangiectasia. Identifying common mutations in each population provides information for genetic counseling, prenatal testing and carrier detection. This study is to permit detection of ATM mutations in Iranian patients. We screened some Hotpoint exons and introns of five A-T patients and their families. Genomic DNA was extracted from their blood samples and the specific regions of the gene were amplified using PCR. Single strand conformation polymorphism (SSCP) was used for mutational analysis of amplified fragments from genomic DNA. We screened 11 terminus exons in which some were overlapped with introns. We identified polymorphic bands on SSCP gels after staining them with silver stain. Among 14 blood samples we studied, 5 were homozygotes for the polymorphism, 5 were heterozygotes, and 4 showed no polymorphism compared to negative control bands. In this study, polymorphism has been identified in exons 40, 41, 58, and 62. Homoallelic mutations would be expected to occur in consanguineous population, whereas heteroallelic (compound) mutation should be frequent among unrelated individuals.

**THE SPECTRUM OF ALPHA-THALASSEMIA MUTATIONS IN IRAN.** *M. Garshasbi Safi Abadi*<sup>1,2</sup>, *H.Y. Law*<sup>3</sup>, *M. Neishabury*<sup>2</sup>, *C. Oberkanins*<sup>4</sup>, *K. Kahrizi*<sup>2</sup>, *E. Keyhani*<sup>2</sup>, *Sh. Dadgar*<sup>2</sup>, *R. Kariminejad*<sup>1</sup>, *N. Al Madani*<sup>1</sup>, *F. Afrozani*<sup>1</sup>, *W. Krugluger*<sup>5</sup>, *H. Najmabadi*<sup>1,2</sup>. 1) Kariminejad/Najmabadi Genetic and Pathology Center, Tehran, Iran; 2) Genetics Research Center, The Social Welfare and Rehabilitation Sciences University, Tehran, Iran; 3) Genetics Service, KK Womens and Childrens Hospital, Singapore; 4) ViennaLab Labordiagnostika GmbH, Vienna, Austria; 5) Institute of Clinical Chemistry, Rudolfstiftung Hospital, Vienna, Austria.

Alpha-thalassemia (-thal) is one of the most common single-gene diseases in the world. It is caused by a variety of deletional and non-deletional -globin mutations, leading to a reduction or complete absence of gene expression. In this study we have tested 87 Iranian individuals, randomly chosen from a pool of patients with low MCV, low MCH, normal or slightly reduced Hb levels, normal HbA<sub>2</sub>, and negative results in -thalassemia genotyping, for the presence of common -thal mutations. Two single-gene deletions (-<sup>3.7</sup>, -<sup>4.2</sup>), five double gene deletions (--SEA, --MED, --THAI, --FIL, -<sup>20.5</sup>), and five point mutations (Hb Constant Spring, Hb Quong Sze, Hb Pakse, Hb Adana, cd 30 delGAG,) were analyzed by PCR and reverse dot-blot methods, and -thal mutations were identified in 50 cases (57%). The following genotypes were observed: -<sup>3.7</sup>/(25 subjects), -<sup>3.7</sup>/<sub>-<sup>3.7</sup></sub> (10) --MED/ (3), -<sup>3.7</sup>/<sub>-<sup>4.2</sup></sub> (2), -<sup>3.7</sup>/<sub>--MED</sub> (1), -<sup>4.2</sup>/<sub>--MED</sub> (1), -<sup>20.5</sup>/<sub>-<sup>20.5</sup></sub> (2), CS/ (4) and CS/CS (1). In 37 individuals none of these mutations was found. Our study shows that the -<sup>3.7</sup> single gene deletion is a very frequent cause of microcytic, hypochromic anemia in Iran, whereas other globin deletions and point mutations seem to be rather uncommon.

**Mutations of the steroid 21-Hydroxylase Gene among Filipino Patients with Congenital Adrenal Hyperplasia.** *E. Cutiongco*<sup>1,2</sup>, *C.E. Abaya*<sup>1</sup>, *C. Padilla*<sup>1,2</sup>, *B.C. Cavan*<sup>2</sup>, *C.L. Silao*<sup>1,2</sup>, *P. Barut*<sup>1</sup>. 1) Inst Human Genetics, Manila, Philippines; 2) Department of Pediatrics, College of Medicine Philippine General Hospital, University of the Philippines Manila.

Congenital Adrenal Hyperplasia (CAH), an autosomal recessive disorder, is due to defective enzymes involved in adrenal steroidogenesis. Phenotypic manifestations are variable depending on the effects produced by the deficient hormones and by the excess production of steroids unaffected by the enzymatic block. CAH, with ethnic and racial variability, has a worldwide incidence of 1 in 15,000. The incidence in France, Italy, Scotland, New Zealand and Japan ranges from 1 in 10,000 to 1 in 23,000. The Philippine CAH crude incidence of 1 in 6,747 (Philippine Newborn Screening Update, 2002) is higher than what is reported in most populations. More than 90% of all cases result from a 21 hydroxylase (cytochrome P450c21) deficiency involving two 21 hydroxylase genes CYP21, the active gene and CYP21P, a pseudogene. Studies have shown that mutations result from unequal crossover during meiosis leading to complete gene deletions, gene conversion events or to point mutations and have demonstrated differences in the frequency of several gene mutations. Using a previously reported combined differential Polymerase Chain Reaction (PCR) and Amplification Created Restriction Site (ACRS) approach (Lee et al, 1996), direct probing of known mutations in exons 1, 3, 4, 6, 7, 8 and intron 2 of the CYP21 and CYP21P genes among Filipino CAH patients was performed. Of the 12 unrelated CAH patients examined, one (8%) demonstrated two different mutations in her CYP21 gene. Ten of the twelve cases (83%) had only one type of mutation detected. Majority of these cases had a premature splicing error involving nucleotide 656 of intron 2. The determination of the most frequent alleles in our population will facilitate rapid screening for 21 hydroxylase gene mutations. Establishment of a definitive diagnosis, essential in the management and counseling of Filipino CAH cases, can also be made available.

**Towards harmonization of external quality assessment (EQA) schemes.** *E. Dequeker, J.J. Cassiman.* Human Gen/Gasthuisberg O&N 6, Univ Leuven, Leuven, Belgium.

Efforts to assess and improve the quality of the output of molecular genetic diagnostic laboratories have been initiated over the last years. Among these are organizations of EQA schemes, also named proficiency testing. Here we provide an overview of the variety of EQA schemes for molecular genetic testing that are being organized in Europe. Several EU member state institutions and international organizations organize EQA schemes at a national, a regional / multinational, or a large-scale international level. Together, EQA schemes have been organized nowadays in Europe for 19 different genetic diseases. For some disorders, multiple initiatives are being undertaken at different levels. For example, for cystic fibrosis genetic testing is one national scheme, three regional or multi-national, and two international schemes, with a range of participation between 6 and 210 labs. For Duchenne and Becker Muscular Dystrophy, four initiatives are undertaken (involving all levels), with number of participants per scheme ranging between 19 and 27. As a logical consequence of this variation between EQA schemes, it becomes difficult for laboratories to distinguish between the designs, and to judge the added value of participation to one or more specific schemes. National or local schemes are important because samples with region-specific mutations can be selected, and reporting can be done in the local language. On the other hand, the organization of large international schemes is more cost-efficient, allows broad recruitment of participants, can provide a broader evaluation of the state-of-the art of routinely applied methodologies for a specific disease, and is more likely to pinpoint eventual shortcomings in commonly used methods. Given the contribution of EQA schemes to assure high-quality genetic service by diagnostic laboratories (education, monitoring of performance), it is encouraging that different organizations are willing to set-up such schemes. However, no guidelines or requirements have been defined yet for the design or organization of EQA schemes for molecular genetic testing, which could lead to a harmonization of different EQA schemes. Suggestions on how to harmonize the different EQA scheme will be further presented in the paper.

**Genetic testing for familial Mediterranean fever in Austria by means of reverse-hybridization teststrips. C.**

*Oberkanins<sup>1</sup>, A. Weinhaeusel<sup>2</sup>, G. Kriegshaeuser<sup>1</sup>, F. Kury<sup>1</sup>, O.A. Haas<sup>2</sup>.* 1) ViennaLab Labordiagnostika GmbH, A-1110 Vienna, Austria; 2) Children's Cancer Research Institute (CCRI), St. Anna Children's Hospital, Vienna, Austria.

Familial Mediterranean fever (FMF) is an autosomal-recessive, inflammatory disorder characterized by short, recurrent attacks of fever, accompanied by pain in the abdomen, chest or joints, and erysipelas-like erythema. Its most severe complication is progressive amyloidosis, leading to end-stage renal failure. FMF predominantly affects Turks, Arabs, Armenians and Sephardic Jews, with carrier rates reported as high as 1 in 5, but has been observed in lower frequencies throughout the Mediterranean area. It is caused by a number of mutations within the marenostin/pyrin-encoding gene MEFV on chromosome 16p13.3, which differently affect the severity of the disease phenotype and the risk to develop renal amyloidosis. Owing to the rather nonspecific clinical symptoms, molecular genetic analysis significantly improves early and correct diagnosis of FMF, and allows to commence lifelong prophylactic treatment of affected individuals with colchicine. We have developed a simple and convenient reverse-hybridization assay (FMF StripAssay) for the simultaneous detection of the following 12 MEFV mutations: E148Q, P369S, F479L, M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S, R761H. The test is based on multiplex DNA amplification and hybridization to a teststrip presenting a parallel array of allele-specific oligonucleotide probes for each mutation. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours, and hybridization/detection may be carried out manually or essentially automated using existing instrumentation (e.g. TECAN profiBlot). After validation on DNAs of known genotype, the assay was used to investigate 199 Austrian individuals suspected to suffer from FMF. We identified MEFV mutations in 109 patient samples, 80 of which carried two, and the remaining 29 carried a single mutation. Ninety patients tested negative for any of the 12 MEFV mutations. (oberkanins@viennalab.co.at).

**Preconception and prenatal cystic fibrosis carrier screening of African Americans reveals a higher carrier frequency and unanticipated frequencies for specific mutations.** *K. Monaghan<sup>1</sup>, D. Bluhm<sup>1</sup>, M. Phillips<sup>2</sup>, G.L. Feldman<sup>2,3</sup>*. 1) Medical Genetics, Henry Ford Hosp, Detroit, MI; 2) University Laboratories, Detroit Medical Center, Detroit, MI; 3) Wayne State University, Detroit, MI.

It is recommended by ACOG/ACMG that cystic fibrosis (CF) carrier screening be made available to African Americans who are either pregnant or planning a pregnancy. One in 65 African Americans is a CF carrier and the current recommended mutation panel detects 69% of mutations in this race, therefore 1/94 African Americans is expected to have a positive carrier screen. F508 accounts for nearly 50% of CF mutations in this group, with 3120+1GA accounting for another 12%. Between April 2001 and June 2003, we performed carrier screening for 1791 African Americans, testing for at least the 25 recommended mutations. A total of 30 CF carriers were detected (1/60), which is greater than expected ( $p < 0.025$ ). Four mutations were detected which are not part of the recommended panel: G622D, F693L (TG), Q98R, and P140S. The F508 mutation was detected in 47% of carriers. We did not detect any 3120+1GA carriers, although we expected 3 carriers of this mutation. The next most common mutations detected were R117H/7T (13%), G622D (10%), F693L (TG) and G551D (7% each). Although F693L (TG) is classified as a CF mutation, its significance is unclear as F693L (TC), originally reported as a CF mutation, was later re-classified as a polymorphism. G622D is reported as a disease-causing mutation and this has been confirmed by others by functional studies. When considering only the 25 recommended CF mutations, 1/78 African Americans screened in our laboratories were carriers (within the expected range); however, the addition of 4 mutations increased the carrier frequency to 1/60, which is significantly higher than expected. The frequencies of several specific mutations detected were unanticipated, as was the absence of 3120+1GA carriers. The current CF mutation panel is likely to be modified as additional racial and ethnic-specific information regarding CF mutations becomes available. Further studies are needed on the incidence of CF mutations that are not part of the current panel, such as G622D.

**Diagnosis of haploinsufficiency of NSD1 in patients with Sotos syndrome using real-time PCR.** *S. Langlois<sup>1, 2</sup>, S. Young<sup>2</sup>, M. Van Allen<sup>1</sup>, S. Lewis<sup>1</sup>, B. McGillivray<sup>1</sup>, S.L. Yong<sup>1</sup>.* 1) Dept Medical Genetics, Univ British Columbia, Vancouver, BC, Canada; 2) Department of Pathology and Laboratory Medicine, Children's and Children's and Women's Health Center of British Columbia.

Sotos syndrome is characterized by developmental delay, macrocephaly, a distinctive facial appearance, overgrowth, and advanced bone age. In 2002, intragenic mutations and deletion of the NSD1 gene were found to cause Sotos syndrome. Published reports of deletions in patients with Sotos syndrome have relied either on the analysis of polymorphic microsatellite markers or FISH analysis. We present the use of real-time quantitative PCR to rapidly assess the NSD1 gene copy number in patients with Sotos syndrome.

Patients with Sotos syndrome seen in our clinic were invited to participate in the study. Taqman primers and amplification probes corresponding to the first coding exon of NSD1 as well as exon 12 of the albumin gene were used to quantitatively co-amplify their respective genes using an ABI 7000 Sequence Detection System. Comparison of the resulting amplification curves to those obtained from normal controls allowed for the NSD1 gene copy number to be determined according to the Ct method. The analysis of 11 patients is complete. Two patients are siblings and 9, sporadic cases. Four patients including the siblings were found to have a deletion of the NSD1 gene. Patient 1 had significant expressive language delay, macrocephaly, overgrowth, advanced bone age, frontal bossing, bitemporal narrowing, and a downward slant to her palpebral fissures. Patient 2 presented with significant developmental delay and macrocephaly. She had a dolicocephalic head, bifid uvula, height (90th perc.), weight (50th perc.), and advanced bone age at age 5. CT scan showed agenesis of the corpus callosum. Patients 3, 4 (sibs) presented with significant developmental delay, macrocephaly, and dolicocephaly. Analysis of parental DNA is pending on all cases. Our results indicate that real-time PCR allows for a rapid DNA based approach to the identification of NSD1 gene deletions and consequent confirmation of a diagnosis of Sotos syndrome.

**Cystic fibrosis transmembrane regulator mutations in Hispanic Californians with cystic fibrosis.** *M. Kharrazi<sup>1</sup>, O. Alper<sup>2</sup>, S. Young<sup>3</sup>, M. Pearl<sup>4</sup>, S. Graham<sup>4</sup>, F. Lorey<sup>1</sup>, J. Sherwin<sup>1</sup>, L-J. Wong<sup>2</sup>.* 1) Genetic Disease Branch, CA Dept of Health Services, Richmond, CA; 2) The Institute for Molecular and Human Genetics, Georgetown University, Washington, DC; 3) Public Health Institute, Oakland, CA; 4) Sequoia Foundation, La Jolla, CA.

Genetic information is currently lacking to build solid population-based cystic fibrosis (CF) screening programs because a large proportion of CF mutations are still unknown, especially in Hispanics. The discovery of mutation 3876delA with 10% prevalence in a selected group in Los Angeles suggests that major CF mutations in Hispanics exist but have yet to be found. The purpose of this study is to describe the distribution of CFTR mutations found in the California Hispanic CF population using multiple mutation sources and stepped mutation analyses. CF cases were identified from California Medi-Cal claims records, death records, and CF Care Center data. Mutation information was abstracted from clinic records and if unknown, analyzed from stored newborn screening blood spots using a 31-mutation panel (Applied Biosystems) or an 87-mutation panel (Genzyme Genetics). If mutations were still unknown, patients were recruited and blood was analyzed using the 87-mutation panel and temporal temperature gel electrophoresis (TTGE). Mutation information is available on over 300 Hispanic CF patients. To date, 94% have both mutations identified and 6% have only 1 mutation identified. Over 55 different mutations have been identified in this population. F508 comprised 62% of the mutations and 3% remain unknown. The 4 most prevalent mutations are F508, G542X, 3876delA and 3120+1G>A. Eight novel mutations were identified through TTGE: 2289-2295 del7bp ins GT, 3960-3961 del A, 360-365 ins T, 379-381 ins T, 1285-1288 ins TA, 296+2T>A, 124-146 del 23bp, and F1016S. Mutation 1285-1288 ins TA was found on 6 chromosomes in 5 unrelated patients with Mexican backgrounds. Unidentified mutations will be further analyzed using DNA sequencing. These data will be used to determine which CF mutations should be included in screening panels in California where over 50% of births are to Hispanics.

**Artificially Constructed CFTR Mutation Samples for Use in Quality Control and Proficiency Surveys:**

**Development and Pilot Testing.** *M. Jarvis*<sup>1</sup>, *R.K. Iyer*<sup>1</sup>, *L.O. Williams*<sup>2</sup>, *W.W. Grody*<sup>1</sup>. 1) Pathology & Laboratory Medicine, UCLA, Los Angeles, CA; 2) Division of Laboratory Systems, Centers for Disease Control and Prevention, Atlanta, GA.

The paucity of patient-derived mutation-positive clinical samples for molecular genetic testing of many heterozygous or rare disorders could be surmounted if it were possible to construct artificial samples containing mutations of interest that would sufficiently resemble natural human samples to function as acceptable and realistic performance evaluation challenges and quality control reagents for recipient laboratories. Using the cystic fibrosis gene (CFTR) as a prototype, we have devised and executed proof-of-principle experiments designed to generate unique mutant cell lines that could be used for these purposes.

We are using site-directed mutagenesis and targeted homologous recombination techniques to introduce specific mutations into the cystic fibrosis (CFTR) gene of a lymphoblastoid cell line (RGA-1). CFTR mutations G85E and N1303K represent the most 5 and 3 mutations in the ACMG-recommended population-screening panel of 25 mutations, while 1078T represents a mutation that has not yet been isolated and stocked for laboratory community use. Molecular testing and characterization of these mutations has proceeded in several defined steps: analysis of spiked genomic DNA containing various concentrations of plasmids and BACs of interest; analysis of DNA isolated from transient transfections; and analysis of DNA isolated from homologous recombination events. Here, we present data for one product construction method that shows positive results when tested at nine pilot facilities using several assay methodologies. All the pilot labs correctly detected the artificial mutations in both heterozygous and homozygous states using platforms varying from reverse ASO hybridization to microarray technology to sequencing. Thus, this method, which is amenable to scale-up and incorporation of virtually any mutation of interest, shows promise as a practical means of obtaining rare positive mutation controls that resemble real human samples for use in quality assurance and proficiency testing.

**Deletion scanning approaches are needed for routine *RB1* analysis.** C. Houdayer<sup>1</sup>, M. Gauthier-Villars<sup>1</sup>, A. Laugé<sup>1</sup>, C. Dehainault<sup>1</sup>, S. Pagès-Berhouet<sup>1</sup>, V. Caux-Moncoutier<sup>1</sup>, F. Doz<sup>1</sup>, L. Desjardins<sup>1</sup>, J. Couturier<sup>1</sup>, M. Tosi<sup>2</sup>, D. Stoppa-Lyonnet<sup>1</sup>. 1) Institut Curie, Paris, France; 2) INSERM EMI 9006, Rouen, France.

Germ-line mutations of the *RB1* gene are associated with a predisposition to retinoblastoma. Twelve years ago, *RB1* deletions emerged as an important cause of retinoblastoma, but are still poorly documented on large series. Since 2001, we have implemented *RB1* testing on a routine basis using a DHPLC method for point mutation detection, combined with a deletion scanning approach that includes karyotypic and molecular investigations. We report the results of this comprehensive deletion screening of all exons and promoter of *RB1* in 192 unrelated patients (102 bilateral and/or familial cases and 90 unilateral, sporadic cases). We used standard cytogenetic evaluation, FISH analysis using a probe covering the whole gene and quantitative multiplex PCR of short fluorescent fragments (QMPSF) to screen for *RB1* rearrangements. In order to confirm exon deletions, long-range PCR was performed with primers flanking the suspected deleted region. Eighteen deletions were found among 88 mutations, ranging from complete gene deletions to single-exon deletions, which represented 20% of the mutations found in our sample i.e. the highest ratio reported to date. All but one were found in bilateral and/or familial cases. All deletions were found using the QMPSF approach and could be confirmed by FISH in 5 cases of complete *RB1* deletions. Among these cases, standard karyotypic examination identified a large del(13)(q14.2q21.2) found in a otherwise mentally-retarded patient. Our results clearly demonstrated the need for a deletion scanning approach in *RB1* analysis and also prompted us to use cytogenetic examinations as a second-line screening when no mutations were found or, alternatively, as a first-line test when a gross rearrangement was suspected from the patients phenotype. No genotype-phenotype correlation could be deduced since in-frame and out-of-frame deletions resulted in bilateral phenotypes. Moreover, the same deletion led to distinct phenotypes within families, thereby highlighting the role of modifier factors.

**Quality assurance of novel diagnostic technologies: a collaborative effort towards the generation of generic SOPs for DHPLC analysis.** *G. Matthijs<sup>1</sup>, E. Dequeker<sup>1</sup>, E. Schollen<sup>1</sup>, G. Michils<sup>1</sup>, B. Vankeirsbilck<sup>1</sup>, J. Harvey<sup>2</sup>, S. McQuaid<sup>3</sup>, R. van Schooten<sup>4</sup>, E. van den Akker<sup>4</sup>, O. Merle<sup>5</sup>, S. Schrooten<sup>5</sup>, Z. Clark<sup>5</sup>, for the Collaborative Group on Diagnostic DHPLC Quality Assurance (DDQA).* 1) Centre for Human Genetics, Leuven, Belgium; 2) National Genetic Reference Laboratory (NGRL), Salisbury, UK; 3) National Centre for Medical Genetics, Dublin, Ireland; 4) VUMC, Amsterdam, the Netherlands; 5) Transgenomic Ltd, UK.

Working in a clinical diagnostic environment, everybody is aware of the drive to ensure the best quality of service. Laboratory accreditation requirements include the availability of validated protocols and traceable quality control data. Obviously, all laboratories that head towards accreditation are faced with the same tasks. They have to generate and validate Standard Operating Procedures (SOPs) for all their methods. A few European clinical genetic laboratories, in close association with Transgenomic, have undertaken a collaborative project to produce SOPs for WAVE and WAVE MD System operation and maintenance, as well as validated protocols for the genes which are routinely being screened by the participating laboratories. Several aspects of the DHPLC method (e.g. PCR requirements, heteroduplex formation, column and oven calibration and stability, selection of temperatures, buffers and control samples, software) have been dealt with in 3 basic types of SOP: a machine or maintenance SOP, a general DHPLC SOP and several disease gene SOPs. These SOPs have been drafted as generic SOPs, which would be useful for all WAVE Users. We have chosen the analysis of MECP2 (Rett Syndrome) to demonstrate the feasibility of this approach. Eight primer sets, for use with Optimase, and the DHPLC conditions, required to effectively detect the most common mutations, have been validated. The primers and sequences were checked using MutationDiscovery.com. The generic SOPs and the validation protocols will be made freely available. The availability of the validated procedures will make the adoption of the technology and its quality management as simple as possible for others. Also, the general idea is applicable to other novel technologies, platforms, methods and kits.

Program Nr: 1428 from 2003 ASHG Annual Meeting

**Two High Throughput Genotyping Methods for Primary Newborn Screening.** *Z. Lin, J.G. Suzow, J.M. Fontaine, E.W. Naylor.* Neo Gen Screening, Inc., Bridgeville, PA.

Since the introduction of newborn screening for PKU in 1962 biochemical assays have been adapted for use with filter paper blood specimens to identify a number of additional metabolic disorders. The recent introduction of tandem mass spectrometry has expanded the scope of newborn screening through the use of multiple metabolite profiling. The recent development of high throughput molecular methods makes the possibility of primary DNA screening an attractive addition to existing newborn screening programs.

We developed a simple, inexpensive, automation friendly DNA extraction method using common reagents to extract DNA from filter paper blood specimens. This extraction method was adapted for use with a Beckman Coulter core robotic system in order to handle the number of samples in a newborn screening program and was used to set up PCR reactions. The first of the genotyping methods is a DNA microarray based platform for population-based screening. Amplified genomic fragments are immobilized onto the surface of a microarray slide such that each spot represents amplified DNA from one individual. Samples from up to 60,000 individuals can be spotted onto a single microarray. Allele-specific hybridization is used to detect mutant and wild type alleles within each spot. Overall costs for each genotyping are shared by the number of samples spotted on each microarray. The second genotyping method is a melting temperature analysis assay using the Roche LightTyper. Fluorescent labeled probes are added during PCR setup eliminating the need for any post-PCR sample handling step. Analysis is achieved by the difference in melting temperatures between matched and mismatched probe set and template. The system can identify the genotype of 384 samples in 10 minutes after completion of PCR. Both genotyping methods have been validated for the screening of sickle cell disease using a large number of newborn samples run in parallel with isoelectric focusing. Both methods have specificity greater than 99%. The LightTyper has advantages in both time and cost and we believe is the better method.

**Multiple Synthesized Control Mutations Optimize Clinical Test Quality.** *R. Lebo<sup>1,2</sup>, G. Dunphy<sup>1</sup>, M. Quicci<sup>1</sup>, D. Galehouse<sup>1,2</sup>.* 1) Dept Pathology & Lab Med, Children's Hospital Medical Center, Akron, Ohio; 2) Northeast Ohio Univ. Coll. Med., Ohio.

Maintaining highly reliable molecular genetic analyses requires simultaneously testing controls for all possible target sequences. Given total genomic template DNA from a single individual from the species to be tested and sequence-specific primers, PCR amplification can synthesize any characterized sequence. Synthesized control mutant sequences serve as standards to optimize and maintain quality control for any reported mutation or polymorphism no matter how rare or unavailable. First, selected short synthesized mutant or variant sequence primers are used to PCR amplify either adjacent upstream or adjacent downstream sequences from total genomic DNA. Then the synthesized upstream and downstream fragments are amplified together to produce a longer fragment with the mutation in the center. A modified strategy simultaneously synthesizes heterozygous controls. Included among the 31 homozygous mutations synthesized on 18 fragments each >400 bp in length are: (1) all 25 mutations in the core cystic fibrosis panel including four unavailable mutations (2184delA, 1078delT, 1898+1->G->A, and I148T), (2) four mutations on the same exon 11 fragment (G542X, G551D, R560T, and 1717-1G->A), (3) all four reflex test controls, and (4) four additional mutations on a commercial test strip. All 31 synthesized homozygous mutations verified on ASO test strips are tested on three test strips with each patient test run. These PCR synthesized controls demonstrate three principles: (1) placing multiple homozygous mutations on the same DNA sequence, (2) analyzing numerous independent homozygous controls from multiple gene regions simultaneously, and (3) using multiplex controls in multiplex tests optimize quality control while minimizing costly control assays. Addition of DNA from an unrelated organism to dilute PCR products will allow synthesized controls to be used throughout robust PCR assays. Splicing and cloning multiple control sequences will enhance long-term preparation and quality control.

**Simultaneous preconception/prenatal carrier screening for multiple genetic disorders by mass spectrometry (MALDI-TOF).** Z. Wang<sup>1</sup>, J.M. Milunsky<sup>1, 2, 3</sup>, V.A. Stevenson<sup>1</sup>, A. Milunsky<sup>1, 2</sup>. 1) Center for Human Genetics; 2) Dept. Pediatrics; 3) Dept. Genetics and Genomics, Boston Univ Sch Med, Boston, MA.

Preconception and early prenatal carrier screening for common mutations in selected genetic disorders is an increasingly desirable goal. For several genetic disorders, such screening is an already expected standard of care. Traditional analyses such as PCR-RFLP and ASO hybridization, are cumbersome, costly, and prone to false positive/negative results. The matrix-assisted laser desorption-ionization and time-of-flight mutation analysis by mass spectrometry has emerged as an efficacious technology offering increased accuracy and specificity, automation and high throughput. We have examined 2 groups of anonymized women in the preconception/early prenatal period studied in consecutive order of receipt of samples. Group I was constituted by 176 Ashkenazi Jewish women who were tested for a total of 19 mutations in the genes for Tay-Sachs disease, Canavan disease, Gaucher disease, Familial Dysautonomia, non-syndromic deafness (Connexin-26), Fanconi anemia, Niemann-Pick disease, and Bloom syndrome, as well as 41 mutations in the cystic fibrosis (CF) gene. 40 (23%) patients were found to carry at least one mutation, with 41 expected based on carrier frequencies for these genes. Group II was constituted by mostly Caucasian consecutive women undergoing routine CF carrier testing in the preconception/early prenatal period. In addition to CF, we assayed for 7 major mutations in 6 genes (Factor V Leiden, prothrombin, MCAD, Smith-Lemli-Opitz syndrome, and non-syndromic deafness (Connexin-26 and 12SrDNA). The mtA1555G mutation in the 12SrDNA gene was included to identify women at risk of having children susceptible to aminoglycoside-induced deafness. The other disorders have a 1% or greater carrier frequency in the patients studied. Carriers for at least one mutation were determined in 23 (11%) of the 209 women studied. Our purpose was to demonstrate the feasibility of preconception/early prenatal carrier testing simultaneously for 68 mutations in 14 common disorders using multiplex assays and MALDI-TOF. This approach appears to be accurate, practical, cost-effective, and informative.

Program Nr: 1431 from 2003 ASHG Annual Meeting

**Direct molecular haplotyping of R117H and intron 8 (IVS-8) thymidine track polymorphisms of the CFTR gene.**

*G. Pont-Kingdon<sup>1</sup>, C. Miller<sup>1</sup>, M. Jama<sup>1</sup>, A. Millson<sup>1</sup>, E. Lyon<sup>1, 2</sup>.* 1) ARUP Laboratories, Salt Lake City, UT; 2) Pathology Department, University of Utah.

Cystic Fibrosis (CF) is a common autosomal recessive genetic disease affecting 1:2500 Caucasian individuals. The disease is caused by mutation in the Cystic Fibrosis Transmembrane Regulatory (CFTR) gene. More than 1000 mutations in CFTR have been documented and the clinical significance of these mutations varies widely. The missense mutation R117H in exon 4 is considered mild if associated with polymorphisms consisting of 7 or 9 thymidines (7T or 9T) in the 3' region of intron 8. Conversely, if an individual carries the R117H mutation in cis with the IVS-8-5T polymorphism, this individual is a carrier of a severe CF mutation. Recently, following recommendations from the American College of Medical Genetics and the American College of Obstetrics and Gynecology, population screening covering a panel of 25 mutations is offered to expectant couples or those planning a pregnancy. The IVS-8-T track is tested only when the mutation R117H is found. If an individual is heterozygous for both the R117H mutation and the 5T variant, additional testing to establish the cis-trans status of both mutations is required to fully interpret the results. This is typically done by linkage analysis requiring DNA samples from parents or close relatives. To address these cases directly we have developed a molecular method to haplotype the mutations and bypass the need to obtain DNA from family members. The assay combines long range PCR (around 18kb) and allele specific PCR. Products are analyzed by melting analysis of hybridization probes or Oligonucleotide Ligation Assay (OLA). Sixteen R117H heterozygous samples were haplotyped. Five of the six possible haplotypes were found in 4 samples with a 5T allele. The accuracy of the PCR product was determined by the concordance of the result between the molecular method and traditional linkage analysis. Accuracy of the detection methods was established by comparison of results from hybridization probes and OLA.

**High Throughput Mutation Screening For Deafness By Denaturing High-Performance Liquid Chromatography.**

*S.D. Prasad, C.J. Nishimura, E.H. Chang, R.J.H. Smith.* Dept of Otolaryngology, University of Iowa, Iowa City, IA.

Genetic testing is playing an increasingly important role in the diagnosis of hereditary deafness. Although mutation screening can be accomplished in numerous ways, all techniques are not equally accurate, timely or cost effective. We use denaturing high-performance liquid chromatography (DHPLC) and offer complete mutation screening of GJB2 and SLC26A4 in persons with presumed autosomal recessive non-syndromic deafness (ARNSD), and of WFS1 and EYA1 in persons with DFNA6/14 or branchio-oto-renal syndrome, respectively. To determine the sensitivity of DHPLC to detect GJB2 allele variants, a panel of 55 individuals segregating 48 known mutations was analyzed by DHPLC, single stranded conformational polymorphism analysis (SSCP) and direct sequencing. The sensitivity of DHPLC to detect these allele variants was significantly greater than SSCP (98.1% vs 82.6%, respectively;  $p=0.0001$ ), with the detection rate of DHPLC nearly equal to that of direct sequencing. Using a panel of 55 individuals segregating 41 different SLC26A4 mutations as determined by direct sequencing of all exons, we found DHPLC sensitivity for detecting SLC26A4 allele variants to be 100%. Screening methodologies were also established for WFS1 and EYA1 on the DHPLC. To ensure high level accuracy and reliability, and to optimize cost effectiveness and turnaround time, we considered the following parameters, amplicon size, primer position, high-stringency PCR conditions, acetonitrile gradient, partially denaturing temperatures, clean durations, water quality testing, DNA-SEP column reliability testing, column maintenance etc. Although the accuracy of DHPLC nearly equals that of direct sequencing, we have not been able to correlate DHPLC chromatogram profile with mutation type. In our experience, wave profile for a given gene allele variant differs from column to column and even in the same column based on column life and buffer constitution. In total, we have now completed mutation screening on 3242 persons out of which 1022 were completed on the DHPLC. This study was supported in part by RO1-DC02842 (RJHS).

**Deleterious BRCA1/2 Mutations detected in a Portuguese Comprehensive Family Cancer Clinic.** *F. Vaz<sup>1,2</sup>, R. Brandao<sup>2</sup>, I. Povo<sup>2</sup>, P. Machao<sup>2</sup>, I. Duarte<sup>2</sup>, P. Rodrigues<sup>1</sup>, O. Costa<sup>1</sup>.* 1) Family Risk Cancer Department, Portuguese Cancer Ctr, Lisboa, PORTUGAL; 2) Molecular Patobiology Investigational CTR (CIPM), Portuguese Cancer CTR, Lisboa, Portugal.

Prevalence and characteristics of BRCA1/2 mutations is not known in portuguese breast and ovarian cancer families. Ours is a comprehensive program : risk characterisation of families and multidisciplinary approach of high risk women, that includes genetic screening of BRCA1/2 mutations. Criteria for BRCA1/2 screening is: at least 25 % of BRCA1/2 combined probability of mutation as calculated using the BRCAPRO or Myriad Models or male breast cancer in the family. Whole DNA was extracted from 25 affected probands from different families and screened with CSGE. 6 deleterious mutations have been found so far (4 in BRCA1 and 2 in BRCA2), and two of these (in BRCA1) are novel:1) del555A in exon 8 leads to a stop codon in exon 11 and a truncated BRCA1 protein; 2)another mutation occurs in a site of alternate splicing in the intronic region of exon 7 and may lead to deletion of a yet undetermined region between exons 8-10. Other BRCA1 mutations are:the 330 A>G and exon 13 duplication. The first is a missense mutation at the donor splice site of exon 5,considered to be a spanish founder mutation of galician origin (Vega A et al Hum Mutation 2001:17:520). Our family is the first without known spanish ancestors (at least for four generations). Exon 13 duplication,an important BRCA1 variant,was found in a family with high probability of BRCA1 mutation but CSGE negative, making it the second portuguese family described with this mutation.

Two BRCA2 mutations,6696del TC and D596H, were found in male breast cancer probands. These were already described in several families of western european origin.

Conclusions: genetic screening is ongoing and is likely that with our strict selection criteria we will obtain a high mutation detection rate.

**Novel Trp3985Arg mutation of the ryanodine receptor (RYR1) gene in a Taiwanese patient with malignant hyperthermia.** *S.I. Hung<sup>1</sup>, J.K. Cheng<sup>2</sup>, Y.L. Lin<sup>1</sup>, J.Y. Wu<sup>1</sup>, Y.T. Chen<sup>1</sup>*. 1) National Genotyping Center, Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Department of Anaesthesiology, Mackay Memorial Hospital, Taipei, Taiwan.

Malignant hyperthermia (MH) is an autosomal dominant pharmacogenetic disorder that causes a life-threatening increase in body temperature after the susceptible individual exposures to certain anaesthetic agents. With a mortality rate of about 10%, MH is estimated to occur in 1 in every 50,000 adult anesthetics. Although the disease is genetically heterogeneous, genotyping analysis is the most effective way for diagnosis of malignant hyperthermia susceptibility. Here, we report the identification of a novel mutation of the intracellular calcium-ion-release channel (RYR1) associated with MH. A 47-years-old female was admitted to hospital for a surgical operation. After a general anesthesia with sevoflurane (concentrations of 2-4 percent), her body temperature rapidly increased to 41.5C. Biochemical exams showed elevated blood creatine kinase (28,485 IU/L). Under the impression of MH, she was supplied with Dantrolene sodium immediately and the clinical symptom subsided gradually. A muscle biopsy with the in vitro contracture test (IVCT) induced by caffeine halothane was performed and showed a positive result. As the skeletal muscle type 1 ryanodine receptor (RYR1) has been reported to be associated with the majority of reported MH cases, the 106 exons of RYR1 gene of the patient were screened by direct sequencing technique. Eleven nucleotide exchanges were found to lead to synonymous amino acid changes; six of them have been deposited in dbSNP database. A nucleotide substitution of T to C at position 11953 which causes a novel amino acid change from Trp3985 to Arg, composing the putative transmembrane domain, was identified at exon 87. Based on this genetic and diagnostic test data, this novel allele, Trp3985Arg, of RYR1 gene was evidenced to be associated with the MH phenotype. In view of the use of genetic method for the diagnosis of MH, the new identified allele should be taken into account.

Program Nr: 1435 from 2003 ASHG Annual Meeting

**Pharmacogenetics: ethical issues.** *S. Thomas.* Nuffield Council on Bioethics, London, United Kingdom.

Pharmacogenetics, the study of genetic variation that affects response to medicines, has the potential to play an important role in improving the safety and efficacy of treatments. However, both the research and its application raise ethical, legal, social and regulatory issues which it is important to consider now. In September 2003, the Nuffield Council on Bioethics will publish its Report on Pharmacogenetics: ethical issues. The talk will draw on the conclusions made in this Report, and present recommendations for future policy and practice. Issues discussed will include the application of pharmacogenetics to the research and development of medicines, the use and storage of genetic information, allocation of resources and the impact on clinical practice. The introduction of pharmacogenetics might lead to a further stratification of the drug market by redefining patient groups. There are concerns that some potentially valuable new medicines may not be developed if, as a result of genetic stratification, the number of patients who would benefit is too small to be profitable. If the potential benefits of pharmacogenetics are to be realised, consideration needs to be given to what incentives should be put in place to maximise possible benefits while also protecting the interests of patients and of society.

**Distribution of serum ferritin and transferrin saturation levels among Asians living in the U.S. and Canada: Hemochromatosis and Iron Overload Screening (HEIRS) Study.** *E.L. Harris<sup>1</sup>, C.E. McLaren<sup>2</sup>, P.C. Adams<sup>3</sup>, G.D. McLaren<sup>2</sup>, T.M. Vogt<sup>1</sup>, B.G. Mellen<sup>4</sup>, M. Speechley<sup>3</sup>, D.M. Reboussin<sup>4</sup>, R.T. Acton<sup>5</sup>.* 1) Kaiser Permanente Center for Health Research (KP), Portland, OR, Honolulu, HI; 2) University of California, Irvine (UCI), Irvine, CA; 3) London Health Sciences Centre (LHSC), London, Ontario; 4) Wake Forest University, Winston-Salem, NC; 5) University of Alabama at Birmingham, Birmingham, AL.

Little information is available about iron overload (IO) or hereditary hemochromatosis (HH) among individuals of Asian heritage. The *HFE* C282Y allele is rare. The HEIRS Study, a multi-site study of genotypic and phenotypic screening for HH and IO in primary-care populations, specifically recruited individuals of Asian heritage in 3 of its 5 Field Centers: KP, LHSC (London and Toronto), and UCI. Screening results from the first 50,000 participants confirm that *HFE* C282Y allele is rare among Asians. However, Asians have the highest prevalence of elevated serum ferritin (SF) and transferrin saturation (TS) levels (TS>50% and SF>300 g/L for men, TS>45% and SF>200 g/L for women). To better understand this finding, we compare TS and SF distributions for 5,086 Asian and 12,071 non-Hispanic Caucasian HEIRS Study participants who do not have *HFE* C282Y or H63D alleles.

Among Asians 7.0% of men and 3.0% of women have elevated TS and SF levels, compared with 1.6% and 0.6% of Caucasian men and women. Mean TS and SF levels for Asian men and women are significantly higher than in Caucasians, adjusted for age and Field Center. Plots of residuals (difference between observed and predicted values) from regression analyses suggest that there may be several underlying SF and TS distributions. Preliminary results from mixture modeling indicate that there are multiple underlying distributions of TS for each race/gender group, and that the means and frequencies of these underlying distributions differ by race and gender. The etiology and clinical significance of these differences is currently unknown. Future HEIRS Study analyses include further evaluation of participants with elevated SF and TS, and gene discovery for non-*HFE* iron overload.

**Genetic susceptibility for lung cancer: interactions with gender and smoking history and impact on early detection policies.** *O. Gorlova*<sup>1</sup>, *C. Amos*<sup>1</sup>, *C. Henschke*<sup>2</sup>, *L. Lei*<sup>1</sup>, *M. Spitz*<sup>1</sup>, *Q. Wei*<sup>1</sup>, *X. Wu*<sup>1</sup>, *M. Kimmel*<sup>3</sup>. 1) Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX; 2) New York Presbyterian Hospital-Weill Cornell Medical Center, New York, NY; 3) Rice University, Houston, TX.

**Objectives:** To identify a genetically high-risk group to develop lung cancer, estimate the lifetime probability of the disease in high risk current, former, and never smokers (males and females), and assess a mortality reduction that can be achieved by screening the high-risk group. **Methods:** Case-control data (764 cases and 677 matched controls), on mutagen susceptibility and DNA repair capacity in different smoking categories were used to obtain the estimates of susceptibility using the formula of total probability. The estimates were inserted into a model of lung cancer natural history and detection by screening to assess mortality reduction due to screening of high-risk individuals. **Results:** The high-risk group included those 12.5% of the population who are above the third quartile for the bleomycin sensitivity and below the median for the DRC. High-risk male current, former, and never smokers have susceptibility to lung cancer of 38%, 21%, and 4%, respectively. Females have lower probabilities to develop lung cancer: 15%, 8%, and 1.5% for high-risk current, former, and never smokers, respectively. Screening of high-risk smokers (12.5% of all smokers) can reduce overall mortality by 7% compared to 30% reduction if all smokers are screened. Analogous results were obtained for former and never smokers. **Conclusions:** Genetic susceptibility constitutes an important factor in selection of high-risk group for early lung cancer detection.

Program Nr: 1438 from 2003 ASHG Annual Meeting

**Media Representations of Genetic Discoveries: What About Headlines?** *T. Caulfield, T. Bubela.* Health Law Inst, Univ Alberta, Edmonton, AB, Canada.

Science and medical stories have to compete with other news stories. Newspapers must therefore offer headlines that are interesting not only to the potential reader, which is the overriding objective of any journalist, but also to colleagues and to management that decides whether to publish a story and where to position it in the paper. However, sensationalism may have a number of effects. For example, in the case of hyped headlines of gene discoveries, one concern is that repeated exposure of the lay public to such headlines may lead to heightened genetic determinism (see Condit, 2001). However, there is, in fact, little data on the accuracy of headlines in relation to stories about human genetics. This poster will present data from an analysis of 590 headlines from 627 newspaper articles reporting on 111 papers in scientific journals. The newspaper stories were limited to those reporting a "genetic discoveries." We used a standard questionnaire and project nave coders. We compared 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 and headlines. We found that the majority of headlines were categorized as having no exaggerated claims (64.2%) or slightly exaggerated claims (24.9%) when compared to the newspaper article. However 10.8% of headlines were moderately-highly exaggerated when compared to the newspaper article. Twice as many headlines (20.9%) had moderately-highly exaggerated claims when compared to the scientific journal article. By comparison, 11% of newspaper articles were categorized as having moderate-highly exaggerated claims and 26.3% had slightly exaggerated claims when compared to their scientific journal article sources (Bubela and Caulfield, submitted).

**The interaction between universities and industry in Genetics The Discovery of BRCA1 and BRCA2 Genes.** *R. Dalpe*<sup>1</sup>, *L. Bouchard*<sup>2</sup>. 1) Dept Political Sci, Univ Montreal, Montreal, PQ, Canada; 2) Dept Sociology, Univ Ottawa, Ottawa, ON, Canada.

**Objectives:** This paper addresses the new dynamics in genetics generated by industry's direct relations with university. (1) to understand the research dynamic occurring between university and industry, and its effect on university research. (2) (exploratory) to analyse the role of researchers and the medical community in the diffusion of new techniques emerging from commercialization. **Theoretical background:** (1) According to sociology of science, a new research system, from disciplinary and curiosity-driven to transdisciplinary and problem-driven research, is established which channels university culture (Gibbons et al, 1994). (2) For sociology of organizations, direct interactions fostered by new institutional arrangements, lead to a more rapid exploitation of new knowledge and the direct role of researchers and universities in commercialization (Powell and Owen-Smith, 1998). Industry may impose constraints, such as in information diffusion to secure property rights. **Methodology:** A case study of the discovery of two genes associated with breast and ovarian cancers was conducted. A bibliometric database of 800 publications has been built to trace the evolution of research between 1993 and 1998 (VanRaen, 1998). The analysis is also based on in-depth interviews of eleven researchers (from five countries) exemplifying the different profiles (Kvale, 1996). **Results:** The new research dynamic foreseen by sociology of science is only in its infancy and national differences remain important. Some actors in large networks are more important than anticipated, for instance the broad medical network was a key player in the diffusion of clinical applications. In scientific research per se, interaction with a clinic was also important. Intellectual property was a major issue and several organizations filed patents to protect and commercialize their discoveries. Our data suggests that scientific collaboration is more difficult when intellectual property is at stake. **Conclusion:** Sociology of networks offers a more accurate analysis of the research dynamic in genetics. The medical community tends to play an important role in the diffusion of the medical innovation.

**Obtaining informed consent from next-of-kin for research using genetic testing: Legal vs. biological relatives.** A. Fishbach<sup>1</sup>, M.A. Austin<sup>2,1</sup>, W. Burke<sup>2,1</sup>, D.J. Bowen<sup>1,2</sup>, T. Vu<sup>1</sup>. 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) University of Washington, Seattle, WA.

In an ongoing study of BRCA2 gene mutations among pancreatic cancer cases enrolled in the Northwest Cancer Genetics Network Registry, we identified a registry participant who had provided a DNA sample, but who had died before consent for this study could be obtained. The IRB approved protocol states that genetic testing can be performed on a deceased participants sample only after obtaining consent from the next-of-kin. The following hierarchical scale for next-of-kin contact, used by many research studies and established through legal precedent, was used: (1) Court appointed guardian, if one exists (2) Durable Power of Attorney, if it specifically includes this kind of authority (3) Legal spouse (one to which community property laws apply) (4) Children 18 & over, if unanimous agreement when >1 (5) Parent or parents, if unanimous agreement if both living (6) Adult siblings, if unanimous agreement when >1. Study staff contacted the legal spouse, who was estranged from the surviving biological relatives. After repeated contact attempts, the spouse refused permission to use the sample. After careful review and consideration given to the potential testing options and health benefits for the biological relatives if a positive genetic test result were obtained on the proband, the IRB granted an exception to the usual next-of-kin contact hierarchy. This decision allows use of the sample based on the biological relatives permission. As a result of this experience, we propose that the legal, hierarchical next-of-kin scale be revised for genetic research, in order to give priority to biological relatives who may benefit from genetic testing. Such proposed scale could use the following criteria: (1) Children 18 & over, if unanimous agreement when >1 (2) Parent or parents, if unanimous agreement if both living (3) Adult sibling, if unanimous agreement when >1 (4) Grandchildren 18 & over, if unanimous agreement when >1. The potential benefits to biological relatives that may result from a genetic testing protocol merit re-evaluation of the paradigm governing next-of-kin contact in genetic research.

**2003 Survey of medical geneticists: Demographics and professional activities.** *M. Blitzer<sup>1</sup>, J. Cooksey<sup>1</sup>, K. Kissam<sup>1</sup>, J. Benkendorf<sup>1</sup>, G. Forte<sup>2</sup>, M. Beaulieu<sup>2</sup>, E. Salsberg<sup>2</sup>.* 1) Univ Maryland Sch Medicine, Baltimore, MD; 2) New York Health Workforce Center, SUNY Albany, NY.

This study was part of a three-year national assessment of genetic services and the health workforce. It was designed to provide current data on the patient care and laboratory services provided by medical geneticists and to answer questions related to their supply and distribution, professional activities, and opinions on various aspects of genetic services. In early 2003, we conducted a mailed survey of all ABMG diplomates certified before 2002. The 67-question survey asked about demographics, education, professional parameters, job satisfaction, and opinions on genetic services. Separate sections asked patient care geneticists about their scope of practice, clinic staffing, and referrals. Laboratory geneticists were asked about laboratory type (molecular, cytogenetics, biochemical), scope of laboratory services, staffing, and referrals. Descriptive statistical analysis was conducted using SAS. The overall response rate was 56% (861/1524). Findings from 765 respondents professionally active in the US are described. Fifty percent were women; mean age was 52 years; and few respondents represented members of racial and ethnic minority groups (10% Asian American, 4% Hispanic, Black, or other). Primary work settings were: academic medical centers (62%), commercial labs (10%), hospitals (9%), medical practices (7%), and 12% other. Mean work levels were 52 hours per week and 48 weeks per year. Geneticists who provided patient care saw, on average, 8 new patients and 6 follow-up patients per week; however, patient care was only one aspect of their work week. Among laboratory geneticists, 16% worked in more than one lab. Analyses by primary laboratory type showed differences across labs. A comprehensive understanding of medical geneticists professional roles, referrals, and other perspectives will assist in broader workforce planning efforts that seek to prepare for growing genetic services demand.

**Transitions in Genetics Services Delivery Models (GSDM): Findings from Case Studies of Four Communities.** *J. Benkendorf<sup>1</sup>, J. Cooksey<sup>1,2</sup>, M. Blitzler<sup>1</sup>, D. Lea<sup>3</sup>, J. Mansour<sup>2</sup>, H. Travers<sup>1</sup>, P. Saunders<sup>4</sup>, C. Gordon<sup>1</sup>*. 1) Univ of Maryland Baltimore, Baltimore, MD; 2) University of Illinois at Chicago, Chicago, IL; 3) Foundation for Blood Research, Scarborough, ME; 4) Georgetown University, Washington D.C.

As part of a 3-year national assessment of genetics services and the health workforce we designed a study based on the premises that 1) genetic services are provided in the context of existing health care organization, and 2) changes in GSDM will reflect genetics-specific factors as well as overall changes in health care delivery. This research used a comprehensive approach and the research design follows a longitudinal study of health systems change ([www.hschange.org](http://www.hschange.org)). We conducted in-depth case studies of genetic services in four geographically diverse communities: Cleveland OH, Miami-Dade County FL, Orange County CA, and Boston MA. Our study used as core data telephone interviews with about 40 respondents in each community selected to cover specific service settings, categories, and types of personnel (geneticists, genetic counselors, physicians, laboratory directors, nurses, state health department representatives, and other health professionals). All relevant data were coded and analyzed using QSR N6 software for qualitative data analysis. Our preliminary analysis shows evidence of transitions in organized GSDM in most major service categories (prenatal, pediatric and adult genetics, laboratory services, state sponsored programs) due to market competition, funding changes, institutional reorganization, and other factors. As examples, AHCs lost market share of genetic testing services and prenatal diagnostic services to commercial laboratories and to free-standing diagnostic centers, respectively. Varied factors and trends affected specific professional roles such that pediatric geneticists are seeing larger numbers of adult patients and genetic counselors practice under a widening range of organizational models. Further analysis will allow us to compare findings from these case studies to our survey research of genetics professionals and to better characterize factors influencing changes in service organization.

**Carrier Screening Panels for Ashkenazi Jews: Is More Better?** *J.R. Leib<sup>1</sup>, S.E. Gollust<sup>2</sup>, S.C. Hull<sup>2</sup>, B.S. Wilfond<sup>2</sup>.*  
1) NGB/NINDS/NIH; 2) MGB/NHGRI/NIH.

Genetic carrier screening programs designed for the Ashkenazi Jewish (AJ) population were first implemented in the 1970s for Tay-Sachs disease (TSD). Testing targeted at this population has since expanded to include 10 autosomal recessive diseases, with panels of up to 9 diseases. The marketing of such panels to the AJ population is a valuable service but creates the potential for stigmatization. To determine the characteristics of AJ panels, we contacted 31 unique US laboratories listed on [www.genetests.org](http://www.genetests.org) that provide TSD testing. Four, in fact, did not provide TSD testing, and we identified one laboratory not listed on GeneTests. We collected data from 27 laboratories that provide AJ carrier screening. We determined the tests offered, panels offered, price and testing methodology used. The tests included 3 diseases associated with death in early childhood (Canavan disease, Niemann-Pick type A and TSD), 5 with a variably shortened life span (Bloom syndrome, cystic fibrosis, familial dysautonomia, Fanconi anemia type C, mucopolysaccharidosis type IV), and 2 diseases that do not routinely shorten the lifespan (Gaucher disease type I and hearing loss). Five diseases lead to progressive neurodegeneration, 2 instill a predisposition to malignancy, 2 result in reduced fertility, and 2 manifest with dysmorphic features. The carrier frequencies ranged from 1/13 to 1/127. All diseases occur more frequently in Ashkenazi Jews except for cystic fibrosis and hearing loss. Panels included from 2 to 9 diseases and ranged in price from \$200 to \$2082. Twenty-one laboratories provided panels. Of these, 18 panels cost less than if the tests were ordered individually, offering an incentive to purchase multiple tests. The defining feature of a disease as being Jewish and inclusion in a panel is the presence of a founder mutation, rather than absolute or relative frequency, or severity of the disease. Public policy guidance for establishing appropriate criteria for inclusion in panels may be useful to laboratories and to the AJ population. Pricing strategies that do not promote more tests should be considered. Developing culturally sensitive educational materials may limit the potential for stigmatization of carriers, affected individuals, and the community.

Program Nr: 1444 from 2003 ASHG Annual Meeting

**Factors affecting social and political legitimacy of rDNA research and embryo science in America and Britain.**

*M.A. Weed, M.R. Seashore, K.K. Kidd.* Dept. of Genetics, Yale U. School of Medicine, New Haven, CT.

Interviews with scientists, advocates and legislators have clarified aspects of policy making on "controversial research". Examples of the effect that different legislative and regulatory environments have on the legitimacy of controversial science are found in the 1970s rDNA research debate in the U.S. and recent action on embryo science in America and Britain. That 95% of private sector scientists complied with guidelines developed by the RAC for federally funded rDNA research demonstrates the regulatory legitimacy that it developed in America. Interviews with British scientists and transcripts of parliamentary debates on expansion of the purposes for which embryo research can be done indicate the development of strong regulatory legitimacy by the UK's Human Fertilisation and Embryology Authority (HFEA). The HFEA has become a guarantor that whatever embryo research is carried out is "necessary", much as the RAC assured Congress that rDNA research was "well regulated". The success of the RAC and HFEA suggests that outside regulation could be essential in the eventual legitimization of embryo research, gene transfer and genetic testing in America. Opponents to "controversial" research have been determined in their efforts to see it slowed or stopped. Interviews indicate that British opponents to embryo research feel that disorganization and limited resources have reduced their political effectiveness in comparison with that of their peers in America. For instance, Parliament and the HFEA have legitimized embryo research in the UK whereas Congress has been less supportive. Media reports suggest that American opponents of regenerative medicine have weakened their enemies by withdrawing financial support from groups with pro-research agendas like the American Heart Association. Proponents of embryo research claim that the Coalition for the Advancement of Medical Research (CAMNR) in America and Association of Medical Research Charities (AMRC) in Britain have been essential in achieving legislative goals like Parliament's passage of permissive regulations on embryo research in 2001 and the failure of the "Human Cloning Prohibition Act" in Congress last year.

**BRCA1 Polimorphisms in Portuguese Breast/Ovarian Cancer Families.** *R. Brandao<sup>1</sup>, P. Machao<sup>1</sup>, I. Pova<sup>1</sup>, I. Duarte<sup>1</sup>, O. Costa<sup>2</sup>, F. Vaz<sup>1,2</sup>.* 1) Molecular Pat. Invest., CIPM , Portuguese Cancer Inst, Lisboa, Portugal; 2) Family Cancer Risk Dep, Portuguese Cancer Institute, Lisboa, Portugal.

25 probands from high risk breast ovarian cancer families were selected for BRCA1/2 screening. So far 6 deleterious mutations have been found, 4 in BRCA1 (330 A>G, del55A, exon 13 duplication and a splicing mutation in the intronic region of exon 7) and 2 in BRCA2 (6696delTC and D596H). Although screening is not yet complete, several polimorphisms, mainly in BRCA1 were observed, some with striking frequency in these probands: IVS8-34 T/C (in 3 cases and one control); 2430T>C (3 cases and one control); the IVS 18+66 G>A (8 cases and 1 control) and IVS6+26invCA (2 cases and one control). Other polimorphisms were:IVS3-212T/C (2 cases) and IVS7+52T>C (2 cases); 1000 A>G and 3667 A>G (one case each). IVS18+66 G>A and 2430 T/C are known polimorphisms shared by several western populations. However IVS-34 T/C and and IVS6+26invCA (this one striking in the fact that was always observed as an homozigous mutation) are not yet described and their significance deserves further study. These findings also account for the sensitivity of our screening methodology (Conformational Sensitive Gel Electrophoresis).

**Prothrombin polymorphism and Lung Cancer.** *K. Yanamandra<sup>1</sup>, J. Rodriquez-Paris<sup>2</sup>, M. Smith<sup>2</sup>, D. Napper<sup>1</sup>, T.F. Thurmon<sup>1</sup>, S.A. Ursin<sup>1</sup>, H. Chen<sup>1</sup>, R. Dhanireddi<sup>1</sup>, J.A. Bocchini Jr.<sup>1</sup>, G.M. Mills<sup>2</sup>, J. Glass<sup>2</sup>.* 1) Dept Pediatrics, LSU Medical Ctr, Shreveport, LA; 2) Feist-Weiller Cancer Center, LSU Medical Center, Shreveport, LA.

Prothrombin is encoded by a 21 kb long gene localized on chromosome 11, at 11p11-q12. The gene is organized in 14 exons, with 5upstream and 3UT regions. Rogier Bertina group (Poort SR et al 1996) from Leiden, the Netherlands, has first identified a polymorphic marker, G20210A in the 3untranslated region of prothrombin gene. The 20210A allele was found to be a common variant and was associated with an increased risk of venous thrombosis. Later, several polymorphic gene factors such as Factor V Leiden, Prothrombin (FII) G20210A, and Methylene tetrahydrofolate reductase (MTHFR) C677T were implicated in hereditary thrombophilia. Prothrombin 20210GA genotype frequency was found to be 1-2% in non-Hispanic white Americans, negligible in Orientals, and less than 0.2-0.3% in African-Americans. This polymorphism was not studied in cancer etiology. In the present investigation we began to study the association of thrombophilic polymorphic gene markers in the etiology of cancer. We genotyped over 200 cancer patients and over 2300 controls. We have not found any mutant 20210AA homozygotes as expected from the literature. However, after stratification by ethnicity and gender we found a significant increase of mutant 20210GA genotypes in Caucasian lung cancer patients than in Caucasian controls 8.7% vs 1.3% (Chi Sq 3.8,  $p < 0.05$ , odds ratio 6.6) and 6.4% in breast and lung cancer patients, and 1.9% in controls (Chi sq 5.5,  $p < 0.02$ , odds ratio 3.6). Based on our pilot data we believe that prothrombin 20210GA genotype is a significant risk factor in the etiology of cancer especially lung cancer. This being the first report in the literature, experience from other centers would definitely strengthen our initial findings. Detailed data and statistics will be presented.

**Identification and detection of mutations causing Niemann-Pick type A Disease in Jewish Ashkenazi population by PCR-RFLP.** *V. Adir, E. Shahak, Z.U. Borochowitz.* The Simon Winter Institute for Human Genetics, Bnai-Zion Medical Center, Technion-Rappaport Faculty of Medicine, Haifa, Israel.

Type A Niemann-Pick disease (NPD) is an autosomal recessive fatal disorder, which is common among Ashkenazi Jews with an estimated frequency of 1:30,000. NPD is caused by a deficiency in the activity of a lysosomal hydrolase, acid sphingomyelinase (ASM) that leads to accumulation of sphingomyelin (SM). The accumulation of SM in ganglion cells leads to cell death.

In an effort to screen large populations of potential NPD carriers, we developed a simple, sensitive and non-radioactive assay. The method developed is based on the use of PCR mediated site directed mutagenesis (PSDM) followed by restriction enzyme digestion, for the detection of the three mutations (R496L, fsP330, L302P), which are responsible for 95% of the type A NPD among Ashkenazi Jews, and for the detection of type B NPD mutation delR608.

Screening of 1189 Israeli Ashkenazi Jews for the R496L, fsP330, L302P using this method revealed carriers at a frequency of 1:79.

Our results show higher carrier frequency and a slight difference in the mutations distributions in the Ashkenazi Jewish population screened at our center, from those described by Schuchman et al, 1997. Similar results were obtained for Tay-Sachs carrier frequency and mutation distribution (Bach 2001, Petersen et al., 1983). The reason for this distinction may be attributed to differences in the populations screened. While both populations come under the global heading of Ashkenazi Jews, the actual composition of the Israeli population may be significantly more homogeneously Ashkenazi Jewish than that of the North American population (Schuchman et al, 1997). This is compounded by the fact that relevant background information is obtained during screening solely by the declaration of the individual tested, which may lack information on non-Ashkenazi Jewish heritage.

**Confidential enquiry into pre-natal genetic care.** *I. Nippert*<sup>1</sup>, *C. Vogel*<sup>1</sup>, *J. Horst*<sup>2</sup>, *B. Eiben*<sup>3</sup>, *F. Louwen*<sup>4</sup>, *P. Miny*<sup>5</sup>, *H. Neitzel*<sup>6</sup>, *J. Schmidtke*<sup>7</sup>. 1) Women's Health Research, Universitaetsklinikum Muenster, Muenster, Germany; 2) Institut fuer Humangenetik, Universitaetsklinikum Muenster, Muenster, Germany; 3) Institut fuer Klinische Genetik und Zytologie Nordrhein, Oberhausen, Germany; 4) Klinik fuer Gynaekologie und Geburtshilfe, J.W. Goethe-Universitaet, Frankfurt, Germany; 5) Universitaets-Kinderspital beider Basel, Basel, Switzerland; 6) Institut fuer Humangenetik, Charité, Medizinische Fakultät der Humboldt-Universitaet zu Berlin, Berlin, Germany; 7) Institut fuer Humangenetik, Medizinische Hochschule Hannover, Hannover, Germany.

**Introduction:** Confidential enquiry is an accepted form of clinical audit that scrutinizes factors which may influence quality and outcome of care. Genetic prenatal diagnosis is part of routine ante-natal care for women with a known a priori risk for a genetic disorder or for those who are identified to be at increased risk by maternal serum screening and/or fetal ultrasound. This study was undertaken to assess how obstetricians manage the genetic aspect of care for these women. **Methods:** The study was designed to investigate the antecedents and sequelae of positive cytogenetic and molecular tests and of positive ultrasound findings. A total of 19.334 pregnancies were followed at different health care settings including university hospitals and private practice. **Results:** The majority (76%) of women with positive test results were offered qualified pre-test genetic counseling although major differences were observed at different settings ranging from 59%-92% of women who received counseling. However only 29% of all women received qualified post-test counseling; again significant differences (17%-100%) in different settings were observed. Termination of pregnancy took place at an average of 3.8 days after the information of the test result was provided (range: 0-13 days). 86% of all terminations took place until 24th week of gestation (overall range: 11+6 32+3 weeks). **Conclusions:** Whilst genetic testing is increasingly offered in pre-natal care, only a minority of women undergo qualified genetic counseling after a positive test result. (The study was funded by the German Human Genome Project.).

**Cost effectiveness of prenatal genetic and ultrasound screening for Van der Woude syndrome.** *G.L. Wehby<sup>1</sup>, R.L. Ohsfeldt<sup>1</sup>, J.C. Murray<sup>2</sup>.* 1) Health Management and Policy, University of Iowa, Iowa City, IA; 2) Department of Pediatrics, University of Iowa, Iowa City, IA.

**OBJECTIVE:** To evaluate the cost effectiveness of genetic screening (GS) and ultrasonography (US), US alone, and no screening (NS), as strategies for prenatal detection and prevention of Van der Woude Syndrome (VWS). **METHODS:** A decision analysis model was used with a time horizon of one generation of gene inheritance and assuming a payers perspective. Three risk groups of parents, differentiated by the probability of having a VWS mutation, were included in the analysis: first-degree relatives of VWS patients, parents diagnosed with nonsyndromic cleft lip and/or palate (NSCL/P), and parents diagnosed with VWS. Effectiveness was defined as having a normal birth outcome. Future costs were discounted to reflect time of their occurrence. Sensitivity analysis was conducted to test the robustness of the results according to the values of parameters used and assumptions made. **RESULTS:** Under reference case assumptions, GS and US had an incremental cost effectiveness ratio (ICER) of \$4667 per additional normal birth versus NS in the group of parents who are at a first-degree relative risk of having a VWS mutation. For parents diagnosed with NSCL/P, GS and US had an ICER of \$12382 per additional normal birth versus US alone. For parents diagnosed with VWS, the strategy of GS and US dominated both NS and US alone. **CONCLUSION:** For parents who are first-degree relatives of VWS patients, GS and US strategy dominates US alone and has a reasonable ICER over NS for most model scenarios. For parents diagnosed with NSCL/P, GS and US strategy has a reasonable ICER over US alone. For parents diagnosed with VWS, GS and US strategy dominates both NS and US alone for most model scenarios. Adoption of Quality Adjusted Life Years (QALYs) gained as another measure of effectiveness allows comparing the ICER of the intervention to those of other health interventions to provide better guidance for resource allocation decisions. A further step of the project will involve eliciting preferences over the health states of VWS patients to calculate the QALYs associated with the model outcomes.

**Genetic screening for Hemochromatosis is no less acceptable than biochemical screening.** *C. Patch*<sup>1</sup>, *P. Roderick*<sup>1</sup>, *W. Rosenberg*<sup>2</sup>, *Southampton Liver Epidemiology Unit.* 1) Health Care Research Unit, University of Southampton, Southampton, Hampshire, United Kingdom; 2) Division of Inflammation, Infection and Repair, University of Southampton.

Hemochromatosis, a treatable adult-onset condition of progressive iron overload is amenable to population screening for early case identification. Screening is possible using a biochemical test for iron overload or a genetic test for the common mutations. The possibility that genetic tests may be less acceptable and have greater adverse psychological consequences than other medical tests has been raised in the literature. We sought to compare the acceptability, feasibility and uptake of genetic and biochemical screening strategies for hemochromatosis. *Methods:* We conducted a randomised controlled trial of equivalence comparing biochemical and genetic screening. The sample was 3000 individuals aged 30 to 70 randomly selected from primary care age/sex registers, stratified for age and gender, randomised to screening arm and invited for screening. Psychological assessments of self-rated health, anxiety and depression were made at four time points. *Results:* Uptake of screening was no lower in the genetic arm than in the biochemical arm. There were no statistically significant differences between the two screening arms in the psychological assessments apart from at testing. Participants in the biochemical arm had lower anxiety and depression scores and higher health profiles. The factors that affected the probability of accepting screening were age, gender and social deprivation. Uptake was higher in women, in older age groups and in less deprived areas. *Discussion:* There appeared to be no difference in the acceptability of screening between a genetic and a biochemical test. The widely held assumption that a genetic test will necessarily cause more anxiety or be unacceptable should be investigated further. Uptake of screening was low overall, but highest amongst older women. Males aged between 30 and 50 years, the key target population have the lowest uptake. This will affect numbers of subjects needed to detect significant clinical differences in future screening and prognostic studies.

**Genetic screening for hereditary haemochromatosis in secondary schools: attitudes of the school community in Victoria, Australia.** *A.A. Gason<sup>1,2,3</sup>, S.A. Metcalfe<sup>1,2,3</sup>, M.A. Aitken<sup>1,2,3</sup>, K. Allen<sup>1,4</sup>, M.B. Delatycki<sup>1,5</sup>*. 1) Murdoch Childrens Research Institute, Parkville, Australia; 2) Dept Paediatrics, The University of Melbourne, Australia; 3) Cooperative Research Centre for Discovery of Genes for Common Human Diseases, Australia; 4) Dept Gastroenterology and Clinical Nutrition, Royal Children's Hospital, Australia; 5) The Bruce LeFroy Centre for Genetic Health Research, Genetic Health Services Victoria, Australia.

Community genetic screening programs can be offered in a variety of settings. Genetic screening for hereditary haemochromatosis (HHC) is the subject of significant debate in the literature. Nevertheless it is the best example of a preventable disease for which genetic predisposition testing is available. In Victoria, Australia, genetic screening for HHC is currently available to adults through a pilot program called HaemScreen. However, secondary schools are an alternative to offer genetic susceptibility screening, as carrier testing for Tay Sachs disease and cystic fibrosis are currently successfully offered in this setting. Screening within schools could vastly increase numbers of individuals exposed to both education and opportunities to be tested. Thus, we aimed to determine the attitudes of the secondary school community towards an HHC genetic susceptibility screening program without offering testing. Students, parents and teachers from secondary schools participated in focus group discussions. These data informed the development of a questionnaire to assess attitudes in students aged 16-17, both before and after an educational session in the school. Parents and teachers received written educational material and a questionnaire. Initial results from focus groups stressed the need for comprehensive education of all participants; concerns were raised regarding privacy of genetic information, paternity, employment and insurance. In conclusion, thorough education is essential to inform the school community of all relevant information in a genetic screening program for HHC. Results obtained from this study of community attitudes could be applied to other genetic screening programs and inform future implementation and policy development.

Program Nr: 1452 from 2003 ASHG Annual Meeting

**Exploring the information provision and in-service training on newborn screening in southern Taiwan.** *M.C. Huang<sup>1</sup>, I.C. Lu<sup>2</sup>, C.K. Lee<sup>1</sup>*. 1) Department of Nursing, National Cheng Kung University, Tainan, Taiwan; 2) Department of Nursing, Chung Hwa College of Medical Technology, Tainan, Taiwan.

**Objective:**To explore the information provision and in-service training for the providers on newborn screening (NBS). **Materials and Methods:**Of fifteen obstetric clinics/hospitals in southern Taiwan, twenty-two information providers were interviewed. Using content analysis method, the transcriptions of interviewed data were analyzed to demonstrate the process of information provision on NBS and the types of in-service training for information providers. **Results:**Although most of clinics/hospitals provided the NBS pamphlet to parents after delivery, they did not provide verbal explanation particularly. The information providers were not trained systematically. They often searched relevant information from NBS pamphlets or through their senior colleagues. **Conclusions:**It is crucial to establish a systematic education program for information providers to promote the quality of information provision on NBS. A further study to investigate the information needs of newborns parents is necessary.

Program Nr: 1453 from 2003 ASHG Annual Meeting

**Craniofacial Brazil Project: preliminary results of a long term health program.** *I.L. Monlleó<sup>1,2</sup>, V.L.G.S. Lopes<sup>1</sup>*. 1) Departamento de Genética Médica, Unicamp, Campinas, São Paulo, Brasil; 2) Departamento de Pediatria, Uncisal, Maceió, Alagoas, Brasil.

In order to recognize the localization of centers involved on attendance of craniofacial individuals and to make the access easier for genetic counseling for these individuals and their families, our team is performing a national study about geographic distribution of specialized hospitals, parents associations and clinical genetics services. This is the first phase of a project untitled Craniofacial Brazil Project, that is pioneer in this area in our country. After an extensive research, we detected 836 services, among 385 specialized hospitals, 79 parents association and 372 health professional faculties on treatment and (or) support for these conditions. A structured form was mailed for them, inviting for voluntary adhesion to the project, asking about characteristics of each service and where and how genetic evaluation and counseling are performed. After 1 month, 2,39 % of the correspondence was mailed back for address problems and 9,33 % of the services were favorable to the proposal. From specialized hospitals, 7,79 % were interested on this project, as well as 12,65% parents association, and health professional faculties 10,21%. Until now, no refused were detectable. This first phase of this project is still in course (conclusion deadline will be in August, 2003) and a diary update is performed, which makes premature any divulgation about genetic evaluation and counseling data. We considered this an important step for national health planning.

**Population studies of three X-linked Immunodeficiency Diseases and Genotype-phenotype correlations of PRF1 mutations in Familial Hemophagocytic Lymphohistiocytosis.** *K. Zhang<sup>1</sup>, S. Lee<sup>2</sup>, J. Villanueva<sup>2</sup>, K. Kogawa<sup>3</sup>, J. Sumegi<sup>2</sup>, R.J. Wenstrup<sup>1</sup>, A.H. Filipovich<sup>2</sup>.* 1) Human Genetics, Children's Hospital, Cincinnati, OH; 2) Hematology/Oncology, children's Hospital, Cincinnati, OH; 3) Department of Pediatrics, National Defense Medical College, Tokorozawa, Japan.

Primary immunodeficiencies are a group of diseases that have been increasingly recognized in both children and adults. Learning the molecular etiology of these diseases will improve understanding of the molecular basis of human immune function. Recently, mutation detection has been chosen as the definitive diagnostic criterion for these life-threatening diseases by the Pan-American Group for Immunodeficiency and the European Society for Immunodeficiency, which otherwise can be difficult to define clinically. We have developed four clinical molecular tests for 1) X-linked Severe Combined Immunodeficiency (IL2RG), 2) X-linked Hyper IgM Immunodeficiency (CD40LG), 3) X-linked Lymphoproliferative Disease (SH2D1A), and 4) Hemophagocytic Lymphohistiocytosis (PRF1). Because the SNPs in these genes are largely unknown, we screened 50 normal female (100 X-chromosomes) of the southern Ohio population for variants. The results have helped us to separate benign polymorphisms from the disease-causing mutations. Perforin mutations have been previously reported in approximately 30% of patients with primary Hemophagocytic Lymphohistiocytosis (HLH). We conducted comprehensive investigation of the genotype/phenotype correlations using perforin mutation analysis by direct DNA-sequencing, perforin expression analysis by flow-cytometry, and NK cell cytotoxicity assay of 50 Northern American families with children diagnosed with HLH. Twenty-five out of 50 had bi-allelic mutations in the PRF1 coding region; most of them were confirmed by genotyping of their parents. Thirteen novel PRF1 mutations were identified. Perforin expression in peripheral blood cytotoxic cells was dramatically decreased or absent regardless of the type of PRF1 mutations. Since approximately half of the cases of HLH in Northern American are caused by PRF1 mutations, DNA based screening for HLH affected families is recommended, even if the proband is unavailable.

**Surveying genomic deletions and duplications through the generation of oligonucleotide fingerprints using primer-free amplification.** *M. Dean*<sup>1</sup>, *B. Gold*<sup>1</sup>, *J. Van Ness*<sup>2</sup>, *D.J. Galas*<sup>2</sup>. 1) Lab Genomic Diversity, NCI-FCRDC, Frederick, MD; 2) Keck Graduate Institute of Applied Life Science, Claremont, CA 91711.

The near complete description of human genomic segmental duplications has been achieved through the application of an exact match algorithm on the April 2003 assembly of the human genome. This permits 1) verification of segmental duplications implicated in causing local genomic instabilities and 2) identification of genomic regions that may be susceptible to genomic rearrangement. To document genomic instabilities, we have devised a method in which a set of oligonucleotides are created and linearly amplified from template structures pre-existing in genomic DNA without the use of pre-synthesized probes or primers. The molecular signatures are composed of 6-16mer oligonucleotides that trigger their own exponential amplification. Through the synthesis of probes with adjoining nicking enzyme sites in combination with naturally occurring restriction endonuclease sites, amplification-templates are generated through incubation with a nicking enzyme (N.BstNBI) and a polymerase in an isothermal reaction at 60C. The triggers formed from the linear amplification are then coupled to an isothermal method for exponentially amplifying the triggering sequences in true chain reactions. Triggering and amplification provide a homogenous assay in which  $10^6$ - $10^9$  fold amplification can be achieved in as little as 3 minutes. Read-out is accomplished by mass spectrometry (LC-TOF or MALDI), real-time fluorimetry, or self-amplifying arrays. Because advance knowledge of the sequence of interest is not required to generate fragments de novo from genomic DNA, the method provides a way to create molecular signatures of genomic changes proximal to restriction endonuclease sites. Further, the method does not require the denaturation of the genomic DNA thus dramatically reducing the complexity of single strand DNA that has to be surveyed during the assay. These panels of diagnostic oligonucleotides can also be used to measure gene expression, and create useful biomarkers. The application of this method to deletions on the X chromosome will be discussed.

**Hidden Complexities of the Human Genome Revealed by Unbiased Mapping of Transcriptome.** *P. Kapranov<sup>1</sup>, S. Cawley<sup>1</sup>, S. Bekiranov<sup>1</sup>, H.H. Ng<sup>2</sup>, E.A. Sekinger<sup>2</sup>, D. Kampa<sup>1</sup>, A. Piccolboni<sup>1</sup>, V. Sementchenko<sup>1</sup>, J. Cheng<sup>1</sup>, J. Drenkow<sup>1</sup>, M. Yamanaka<sup>1</sup>, S. Patel<sup>1</sup>, S. Brubaker<sup>1</sup>, H. Tammana<sup>1</sup>, B. Narayanan<sup>1</sup>, G. Helt<sup>1</sup>, K. Struhl<sup>2</sup>, T.R. Gingeras<sup>1</sup>.*  
1) Affymetrix Inc, 3380 Central Expressway, Santa Clara, CA 95051; 2) Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115.

Recent completion of key genomic sequences, hallmarked by those of human and mouse, has provided unprecedented opportunities for the use of high density oligonucleotide arrays to discover novel transcripts, to map functionally important genomic regions and to integrate all this information in order to provide a genome-wide view of the transcriptional regulatory networks in the cell. We have recently reported unexpectedly high levels of transcriptional activity in human chromosomes 21 and 22 (Kapranov et al., 2002). It was shown that up to an order of magnitude more genomic sequence can be transcribed and transported into cytosolic mRNA than previously anticipated based on annotations of known exons in the human genome. This work has led to other ongoing studies involving the use of high density oligonucleotide array technology to empirically detect and map hitherto unknown transcriptional units and functional elements in the human genome. These observations have the potential to alter our current views of a genome's structure, regulation and function.

Program Nr: 1457 from 2003 ASHG Annual Meeting

**In silico prediction of novel neuronal-specific composite regulatory elements.** *S. Keryanov, K. Gardner.* Dept Neurology, Univ Pittsburgh, Pittsburgh, PA.

**Aim:** Composite cis-regulatory elements (CRE) in promoter and enhancer regions via binding with respective transcription factors essential for transcription control of gene expression. Here, our focus was to identify additional modules comprised of CREs for known transcription factors that predict neuronal-specific expression patterns. **Methods:** The approach was illustrated by comparative in silico search and characterization of two promoter regions cloned by our lab for the human KCN10 gene. Double Gn boxes combined with NF-Y and NFAT binding sites (REL factors) were identified exclusively in the distal promoter region which regulates the neuronal-specific isoform but not in the proximal promoter region which regulates the ubiquitously expressed isoform. Secondly, promoter regions of human Na, K-ATPase alpha 1,2,3 (ATP1A1,2,3) genes with known tissue-specific patterns and the putative 5 flank of the ATP1A4 gene were characterized. Multiple GC boxes (binding sites for Sp1/Sp5 proteins) in combination with other factors were typically found in 5 flanks of ubiquitously expressed genes such as ATP1A1. However, the promoter region of the neuronal-expressed ATP1A3 gene contained a double Gn box and NFY/REL factor. **Results:** Promoter and intron 1 in silico analysis of 47 abundantly neuronal expressed genes revealed that ~48.5% sequences contain a combination of Brn-2 / Brn-4 sites and mainly REL factors. Approximately 37.4% contain a double Gn box with only REL factors. The double Gn box is likely to be a target for the neuronal-specific transcription repressor Necdin. This in silico approach might provide a real alternative to predict key elements of neuronal-specific gene expression.

**Genomic structure of segmental duplications on human chromosome 8p23: implications for a modifying risk factor in hearing impairment due to the mitochondrial mutation A1555G.** *E. Ballana*<sup>1</sup>, *N. Bosch*<sup>1</sup>, *N. Fischel-Ghodsian*<sup>2</sup>, *X. Estivill*<sup>1</sup>. 1) Genes and Disease Program, Genomics Regulation Center, Barcelona, Catalonia, Spain; 2) Department of Pediatrics, UCLA School of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA.

A five-Mb region of human chromosome 8p23 is inverted in a significant proportion (about 25%) of individuals of the general population. In addition to this common inversion, chromosome 8p23 hemizyosity and duplication are involved in several disorders. Linkage analysis has identified a putative modifier locus on 8p23 (around marker D8S277) for the hearing impairment phenotype due to the mitochondrial A1555G mutation. A1555G, affecting the mitochondrial 12S rRNA gene, has been associated with non-syndromic deafness with a variety of clinical phenotypes, yielding from mild to severe hearing impairment. The nuclear background is thought to modulate the phenotypic expression of this mtDNA mutation. We have characterized this genomic region and have found that flanking the inversion there are two pairs of segmental duplications with a complex organization. Although the sequence corresponding to the segmental duplications still contains 3 gaps, it is organized in 16 distinct subgroups of segmental duplications. Each subgroup comprises between 2 and 5 duplicons, ranging from 5 to 200 kb and a sequence identity between 93% and 99%, resulting in a mirror image defined by the duplicons that enables the 8p23 region to undergo different rearrangements. Several clusters of genes encoding defensins are located within these segmental duplications. In order to analyse the role of 8p23 genomic architecture as a modifying target for the A1555G phenotype, distinct microsatellite markers and defensin gene promoters located in the region have been screened in families carrying the A1555G change. By combining DHPLC, subcloning and sequencing we have found that both the defensin cluster and microsatellite markers of the region are polymorphic in copy number and sequence orientation. We suggest that this high level of polymorphism may have a role in the age of onset and severity of hearing impairment in patients carrying A1555G.

**Evidence of a founder effect for the 235delC mutation of *GJB2* (connexin 26) in East Asians.** D. Yan<sup>1</sup>, H.J. Park<sup>2</sup>, X.M. Ouyang<sup>1</sup>, A. Pandya<sup>3</sup>, K. Doi<sup>4</sup>, R. Erdenetungalag<sup>5</sup>, L.L. Du<sup>1</sup>, W.E. Nance<sup>3</sup>, A.J. Griffith<sup>2</sup>, X.Z. Liu<sup>1</sup>. 1) Dept Otolaryngology, Univ Miami, Miami, FL; 2) NIDCD, NIH, Rockville, MD; 3) Medical College of Virginia at VCU, Richmond, VA; 4) Dept of Otolaryngology and Sensory Organ Surgery, Osaka, Japan; 5) Dept of Human Genetics, Maternal and Child Health Research Center, Ulaanbaatar, Mongolia.

Mutations in the *GJB2* gene encoding connexin 26 (Cx26) are a major cause of autosomal recessive and sporadic cases of congenital deafness in most populations. The 235delC mutation of *GJB2* is the most frequent known mutation in some East Asian populations, with a carrier frequency of approximately 1%. The 235delC has never been reported in Caucasians. In order to explore whether the high frequency of the 235delC mutation of *GJB2* in East Asian populations is the result of a founder effect or a mutational hot spot, we searched for evidence of a shared common haplotype of 235delC with flanking polymorphisms. We analyzed three non-synonymous polymorphisms within the coding region of *GJB2* (V27, E114G, I203T) and four flanking SNPs in a total of 26 homozygous and 19 heterozygous 235delC chromosomes from Mongolia, China, Korea, and Japan and compared them to panels of 362 controls. In each population, the 235delC mutation was in significant disequilibrium with 5 linked polymorphic markers. In 235delC homozygotes, 235delC was associated with one core SNP2-V27I-E114G-SNP1 haplotype, A-G-A-C. In contrast, four major haplotypes were observed in the normal controls: G-G-A-C and A-G-A-C were the most common with frequencies of 30% and 21%, respectively. The detection of 235delC only in East Asians but not in Caucasians, and the small chromosomal interval of the shared haplotype, suggests that 235delC is an ancient mutation that arose after the divergence of Mongoloids and Caucasians. Similarly, the fact that the 235delC mutation appears on a single haplotype provides no support for the possibility that recurrent mutation is the explanation for the high frequency of the allele.

***In vivo* Characterization of the Transcriptional Response of Kidneys from *Aprt* Knockout Mice to Crystals of 2,8-Dihydroxyadenine.** *J. Chen*<sup>1</sup>, *Y. Chen*<sup>1</sup>, *D. Glass*<sup>1</sup>, *S.B. Bledsoe*<sup>2</sup>, *A.P. Even*<sup>2</sup>, *A. Sahota*<sup>1</sup>, *J.A. Tischfield*<sup>1</sup>. 1) Department of Genetics, Rutgers University, Piscataway, NJ; 2) Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN.

Adenine phosphoribosyltransferase-deficient (*Aprt*<sup>-/-</sup>) mice develop 2,8-dihydroxyadenine (DHA) nephrolithiasis, initially in collecting ducts, but how crystals form in this site is not clear. In this study, we used the Murine Genome Array to identify genes showing altered expression in kidneys from *Aprt*<sup>-/-</sup> mice at 1, 4, and 12 weeks postnatally. From over 12,000 genes, we identified 96, 88, and 85 genes, respectively, which were differentially expressed in these mice. Eight of these genes were common to all three age groups. Of 73 genes up-regulated in 12-weeks-old *Aprt*<sup>-/-</sup> mice, 44 were annotated as immunoglobulins or proteins related to inflammation/fibrosis, which is in full agreement with our previous histopathological observations. Interestingly, there were significant alterations in the expression of several genes previously not known to be associated with kidney stone disease. These included small proline-rich protein 2f (*Sprr2f*), cytokeratin 19, cytochrome P450, cell death activator A, and lysozymes. Real Time RT-PCR was used to confirm the microarray results. These findings demonstrate that DHA crystals modulate the expression of genes involved in several important kidney functions, including barrier fortification, apoptosis, cell injury, and proliferation. Because of its potential role in membrane functions, *Sprr2f* was chosen for further study. *Sprr2f* is a cross linker of cell envelope proteins that is not expressed in normal kidneys; however, it is expressed at high level in 1-week-old *Aprt*<sup>-/-</sup> mice, but not at later stages. Immunohistochemistry analysis showed that this protein is expressed in collecting ducts, the same location where DHA crystal deposition and mitosis are first observed. Water re-absorption at this site may account for DHA precipitation and crystallization. These results imply that *Sprr2f*-induced barrier fortification and proliferation of the collecting duct cells may play important roles in the formation of intratubular DHA crystals and subsequently stones.

Program Nr: 1461 from 2003 ASHG Annual Meeting

**Multiple Objective Linear Programming for Metabolic Networks.** *M. Sun*<sup>1</sup>, *MM. Xiong*<sup>2</sup>. 1) Dept Business, Univ Texas, San Antonio, San Antonio, TX; 2) Human genetics Center, University of Texas Health Science Center at Houston, Houston, TX 77030.

Living organisms have evolved by maximizing their surviving probability or fitness function. In the past several years, maximization fitness function subject to flux balance equations is reduced to a linear programming (LP) problem. However, metabolic networks evolve to accomplish multiple objectives: maximizing biomass production and ATP generations or optimizing consumers of available substrates. Multiple objectives will be fitness functions. The driving forces governing cellular metabolism are to compromise between ATP, redox, and biomass production. Therefore, a single LP is not sufficient to model operations of metabolic networks. To overcome this limitation, we propose a multiple objective LP model for metabolic networks. We will use duality theory to develop algorithms for identifying all efficient solutions to multiple objective LP. We will also perform sensitivity analysis to investigate the impact of addition or removal of biochemical reaction on the performance of metabolic networks and adaptation of metabolic networks in response to changes of various environments. Finally, we will investigate how multiple objectives are compromised to seek adaptive operation or evolution of metabolic networks.

**Nucleotide Intelligence Pattern Statistics in Bioinformatics--- Loci Expression on A Chromosome in Genetics &**

**Application.** *K. Chen*<sup>1, 2</sup>. 1) Visiting Professor Biostatistics and Bioinformatics Unit, Medical Statistics Section, Division of Hematology/Oncology, Department of Medicine, School of Medicine, The University of Alabama at Birmingham; 2) Professor, Department of Laboratory Medicine, NTU School of Medicine; Department of Biostatistics Institute of Epidemiology, NTU School of public Health; National Taiwan University, Taipei, 100 Taiwan.

Nucleotide information of a genome study in bioinformatics is transformed as nucleotide intelligence in the 3 levels of realization: chromosome, locus and gene. A chromosome is identified as an open interval on a real line; a locus on the chromosome is embedded on the open interval as a point to which the coordinate is decided; the gene of the locus on the chromosome of a certain subject is the value for a certain function of the coordinate for the point representing the locus. This 3-level realization takes care of the physical relationship for loci on the same chromosome, the linkage in genetics. The pattern recognition of the functions identifies the pattern of the intelligence. The study employs the established results of representation of a curve-hypersurface or image (CHOI) for data analysis and CHOI pattern recognition in biostatistics. The establishment is from the modeling that integrates the disciplines of bioinformatics, qualitative investigation (QI), biomathematics and biostatistics to define the statistics on CHOIs and to infer the likelihood for hypothesis testing. The study concentrates on employing QI on massive genome information to express as intelligence in terms of a set of CHOIs that are characterized by a limited number of continuous valued parameters. The number of the parameters is so limited that biostatistical inference on the parameters is manageable. The study moves on employing the parameters to characterize, with maximized likelihood estimators, the pattern of the embedded intelligence. A nucleotide information-profile in genetics for a clinic study in medicine stimulates the modeling for various issues in bioinformatics on analyzing DNA data. The functional relationship of loci on different chromosomes is not included here, and will be in the coming study of epistasis.

**Quantitatively prioritizing candidate disease gene sequences using annotation.** *T. Braun*<sup>1,2,5</sup>, *T. Scheetz*<sup>2,4</sup>, *H. Abdulkawy*<sup>5</sup>, *B. Brown*<sup>5</sup>, *S. Davis*<sup>5</sup>, *B. O'Leary*<sup>5</sup>, *J. Ritchison*<sup>5</sup>, *R. Sutphin*<sup>2</sup>, *S. Shankar*<sup>2</sup>, *V. Sheffield*<sup>3</sup>, *E. Stone*<sup>2</sup>, *T. Casavant*<sup>1,4,5</sup>. 1) Dept Biomedical Engineering; 2) Dept Ophthalmology/Visual Sciences; 3) Dept Pediatrics; 4) Center for Bioinformatics and Computational Biology; 5) Coordinated Laboratory for Computational Genomics, University of Iowa, Iowa City, IA.

A common method for mutation identification is to screen each successive candidate gene to completion. This approach is generally favored, and even considered practical, due to the overhead of identifying and acquiring the gene structure and annotation, selecting primers, and managing multiple assay results spanning the transcript and genomic sequence - the results of multiple PCR reactions, SSCP assays, and sequencing results. TrAPSS (Transcript Annotation Prioritization and Screening System) is an integrated system that utilizes sequence annotation features to accelerate the mutation screening process by predicting the potential of gene sub-sequences to contain disease-causing mutations. Evolutionarily conserved functional domains and motifs across species may contribute valuable information for determining the functional significance of gene sequences. This research explores the contributions of several types of annotation. These include: functional domains, secondary structure prediction, similarity to model organism sequences, SNPs, and conserved intronic and regulatory elements. A component of the system is a parallel amplicon prioritization algorithm PAR (Prioritization of Annotated Regions). This parallel amplicon prioritization algorithm is based on the assumptions that mutations are not uniformly distributed and that particular sequence features will be more sensitive to sequence changes. TrAPSS utilizes annotation to prioritize candidate genes and sub-regions of genes. It also distributes screening resources across multiple genes in parallel, while still covering entire genes when mutations are detected. A retrospective study examined 710 genes from OMIM that collectively contained 4,115 published mutations. The PAR algorithm increases the rate of mutation detection with a reduction of screening resources by more than 91%.

**Characterization of LINE 1 sequences on the human chromosome 21 short arm.** *H.D Beris<sup>1</sup>, M.R. Cummings<sup>2</sup>, J.L. Doering<sup>1</sup>*. 1) Dept. of Biology, Loyola University Chicago; 2) Dept. of Biological Sciences, University of Illinois at Chicago.

LINE-1 (L1) sequences are retrotransposons distributed throughout the human genome. There are about  $5 \times 10^5$  copies of L1 constituting 17% of the genome. The majority of these copies are 5' truncated and inactive. The organization of L1s in heterochromatic regions, like that found on the HC21p arm, is not well characterized. Our work using an HC21 hybrid cell mapping panel indicates that most 21p L1 elements are full length (FL), polymorphic in number and organization, with substantially more FL L1s on 21p than 21q. Since most FL L1s are in 21p heterochromatin, this suggests preferential integration sites, or lack of selection against such L1s in this region, resulting in their preferential accumulation on 21p. A number of FL and truncated L1 elements in the HC21 heterochromatic region have been subcloned from a yeast artificial chromosome contig containing portions of the centromere and p arm of HC21. Three subclones, one containing a FL L1 and two containing truncated L1s, have been sequenced and compared to the L1s on 21q with respect to target site duplication (TSD) region, integration site, and 3' transductions. The FL L1 belongs to the L1PA7 subfamily and contains an 8 nt TSD, which is shorter than the TSDs found on most 21q FL L1s. It is not integrated into the consensus AT rich integration site, suggesting integration via an endonuclease independent mechanism. This FL L1 also has two transductions of 81 nt and 16 nt. The truncated L1s belong to the L1PA2 and L1PA4 subfamilies. The finding of an L1PA2 suggests that evolutionarily young L1s can integrate into heterochromatic regions without negative selection and may account for some of the polymorphism seen in the structure of 21p. The concentration of FL L1s on 21p may facilitate p arm interactions among acrocentric chromosomes, increasing the risk of translocations and non-disjunction and may increase the rate of genome evolution.

**A dual role of segmental duplications in evolutionary rearrangements and human genomic disorders. L.**

*Armengol<sup>1</sup>, M.A. Pujana<sup>1</sup>, J. Cheung<sup>2</sup>, S.W. Scherer<sup>2</sup>, X. Estivill<sup>1</sup>.* 1) Genes and Disease Program, Genomics Regulation Center, Barcelona, Catalonia, Spain; 2) Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Ontario, Canada.

An initial comparison between the genomes of human and mouse has identified over 300 segments of synteny, but the nature of rearrangements leading to breaks of synteny (BOS) has not yet been elucidated. While the different segment sizes agree with a random breakage model of genome evolution, a tendency for chromosomal breaks to reoccur at certain genome regions has been suggested. The identification of sequences that are involved in evolutionary rearrangements should provide important insights into the understanding of genome plasticity, and should pinpoint regions that might be associated with disorders that undergo mutations at the genomic level. We have compared the human and mouse genome sequences and demonstrate here that recent segmental duplications correlate with BOS between these two species. By comparing observed and simulated data (390,000 simulations of human chromosome breaks), we found that the co-occurrence of segmental duplications at regions of BOS is significantly different from what one would expect by chance. Thus, 53% of all evolutionary rearrangement breakpoints between these species associate with segmental duplications, as compared to 18% expected in a random location of breaks along the chromosome ( $p < 0.0001$ ). We also observed that nine primary regions involved in human genomic disorders show changes in the order or the orientation of mouse/human synteny segments, and are often flanked by segmental duplications in the human sequence. These findings establish a strong relationship between human genomic disorders and evolutionary rearrangements with the mouse genome, being segmental duplications a common feature of both. Thus, the data presented here could help to the identification of candidate regions to be analyzed for new genomic mutations. Our data suggest that segmental duplications have participated in the recent evolution of the human genome, as driving forces for evolutionary rearrangements, chromosome structure variations, and genomic disorders.

Program Nr: 1466 from 2003 ASHG Annual Meeting

**Enterprise Genealogy: Managing the Life of a Sample.** *J. Ganjei, S. Alavi.* LabVantage Solutions, Inc. Bridgewater, NJ.

The evolution of high throughput technologies to identify and characterize genomic and proteomic data has been a major challenge to information management and bioinformatics. As information is nowadays more web-centric, the problems related to organizing and indexing large amounts of previously unrelated data is moving to the forefront. Thus, LIMS software must evolve to enable high volume processing of such heterogeneous data, irrespective of location and form. A framework for providing definitions, toolkits, and linking of multidimensional data, to facilitate data analysis, is essential, and is discussed below. Central to the overall operation of the laboratory is the integration of experimental protocols and workflow processes. Sapphire, an Enterprise LIMS software solution provided by LabVantage, has been used in many types of laboratory settings to manage samples, experiments, and results, in a systematic and organized fashion. This presentation explores Sapphire's utility to meet the demands of high throughput genomic and proteomic information management, specifically discussing the importance of project study management and hierarchy, experimental management, and integration of analytical and 3rd party tools, to provide a complete genealogy of the data. The core capabilities of Sapphire range from the ability to integrate information from analytical instruments and robotics devices, to reporting tools.

**Use of motif scanning to identify Phosphopantetheinyl transferases in yeast and man.** *M.T. Geraghty<sup>1</sup>, N. Braverman<sup>2</sup>*. 1) Dept Genetics, Children's Hosp Eastern Ontario, Univ Ottawa, ON, Canada; 2) McKusick-Nathans Inst Genetic Medicine, Johns Hopkins University, Baltimore, MD, USA.

Phosphopantetheinyl transferases (PPTs) activate enzymes by transferring the prosthetic group 4'-phosphopantetheine onto a specific target binding site. PPTs are characterized by an amino acid motif **G-X-D-X(2,100)-[FW]-X-X-K-E-[SAC]-X-X-K**. Using this sequence as a probe and the ScanProsite algorithm (<http://us.expasy.org/tools/scanprosite/>), we identified 4 putative PPTs in the proteome of *S cerevisiae*. These include Lys5p which activates -aminoadipate reductase (Lys2p); PPT2p which activates the mitochondrial Acyl Carrier Protein (ACP); cytoplasmic Fatty Acid Synthase (FAS2p) which auto-activates itself and a novel putative PPT, encoded by YDR520C. A similar scan of the human proteome identified 4 candidates; -aminoadipate semialdehyde dehydrogenase-phosphopantetheinyl transferase (AASD-PPT), the alpha and gamma sub-units of an Alcohol Dehydrogenase (P07327 and P00326) and the Emilin-2 precursor (Q9BXX0). Conversely target proteins for the PPTs have a highly conserved amino acid motif which acts as the binding site for 4'-phosphopantetheine, **L-G-[LAYG]-[DH]-S[LI]**. A motif scan of the yeast proteome identified 9 proteins including previously reported target proteins (ACP and Lys2p) and in addition the ADP-ribosylation factors 1 and 2. In the human proteome we identified a small subset of proteins including those orthologous to that found in the yeast search *i.e.* the Acyl Carrier Protein subunit of the NADH-ubiquinone oxidoreductase (NDUFAB1), the human Fatty Acid Synthase and the ADP-ribosylation factors 1 and 3 (ARF1 and ARF3). We have previously shown that the human AASD-PPT gene can complement the yeast *lys5* knockout and correct the lysine biosynthetic defect in this strain. However, man lacks a lysine biosynthetic pathway and it is unclear that AASD-PPT plays a role in the oxidation of -aminoadipate semialdehyde or that there is in fact a human equivalent of LYS2. This study shows that AASD-PPT may not be the only phosphopantetheinyl transferase in the human proteome and that FAS, NDUFAB1, ARF1 and ARF3 proteins are targets for the human PPTs identified here.

Program Nr: 1468 from 2003 ASHG Annual Meeting

**LAG-1 and LD78- chemokine genes are located in a segmental duplication at chromosome 17q12.** *W.S. Modi.*  
SAIC, National Cancer Institute-Frederick, Frederick, MD 21701 USA.

Sixteen CC chemokine genes form three clusters in an interval approximately 1.8 Mb in size at 17q12-21, and 14 of the genes are located on genomic contig NT\_010799. The two genes not on the contig (LAG-1 and LD78-) are 95% similar to two that are on the contig (MIP-1 and MIP-1). LAG-1 and LD78- are about 12 kb apart on clone AC036181, while MIP-1 and MIP-1 are similarly 12 kb apart on clone AC003976. Dot matrix analysis of the two clones reveals 90% similarity between the 40-kb intervals containing these genes and their flanks, suggesting segmental duplication is responsible for the origin for one of the two gene pairs. Additionally, other loci from AC036181 map to 150 different positions in the human genome revealing a very complex pattern of duplication. Recent UCSC, NCBI, and Celera assemblies delete or misrepresent genomic clones containing these two genes, indicating that substantial hand-editing may be necessary to complete the assembly of the human genome sequence in regions such as this containing duplicate genes. This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. NO1-CO-124000.

**Gene expression profiling of bronchoalveolar lavage cells in acute lung rejection.** *J. Lande, V. Gimino, T. Berryman, M.I. Hertz, R.A. King.* Medicine/Inst Human Genetics, Univ Minnesota, Minneapolis, MN.

**Background:** Lung and heart-lung transplantation are effective treatments for many diseases unresponsive to other therapy. However, long term survival of recipients is limited by the development of bronchiolitis obliterans syndrome (BOS). Acute lung rejection (AR) is common and is the leading risk factor for the development of BOS. Despite many investigations there are no reliable biomarkers of AR. We used microarray analysis to investigate the gene expression of bronchoalveolar lavage (BAL) cells to identify biomarkers for AR. **Methods:** Gene expression microarrays were performed using RNA from BAL cells of lung transplant recipients with AR (n=7) or with no rejection (n=27). BAL cell and differential counts were done. Statistical Analysis of Microarrays (SAM) was used to compare signal values between groups. The relationships of transcripts found differentially expressed between the groups were visualized with 2D hierarchical clustering (Cluster, Treeview), and relationships of significant genes across biologic pathways (GenMAPP) were investigated. **Results:** The BAL cell counts were similar between both rejection groups and differentials indicated no significant difference in cell composition between groups. Both groups consisted primarily of macrophages, but with a small percentage of lymphocytes. 135 genes were upregulated in the AR samples as compared to the no rejection samples with a false discovery rate less than 1%. These included AR response genes, immune-response genes with unknown roles in AR, and genes of unknown function. Clustering grouped all AR samples, and separated them from the majority of the no rejection samples. The genes that were differentially expressed in AR samples displayed 6 GenMAPP pathways with significant changes when an expanded list of 885 genes were found using SAM with a false discovery rate less than 5%. **Conclusions:** Microarray analysis is a powerful tool to identify candidate genes involved in acute rejection of the lung allograft. The individual genes, distinct patterns of gene expression, or biologic pathways identified may represent novel biomarkers for AR or for the development of BOS.

**SNP Information Mining Pipeline (SIMP) for Complex Disease Studies.** *C. Liu<sup>1, 2</sup>, T. Nguyen<sup>1</sup>, R. Zhang<sup>2</sup>, F. Yao<sup>2</sup>, Z. Zhu<sup>2</sup>, E.S. Gershon<sup>1</sup>.* 1) Dept Psychiatry, Univ of Chicago, Chicago, IL; 2) National Lab of Medical Genetics of China, Central South University, Changsha, China.

SNPs are the major genetic markers commonly used for association studies, in the hunt for genes responsible for complex/common diseases. A region-wide (~ 10 Mb size genomic region) association study require utilization of hundreds or more SNPs. More than 4 million SNPs were deposited into dbSNP. ~ 6% of them have allele frequency data. ~ 80% of them are mapped by NCBI. There are more than 10,000 SNPs in a region of ~ 10 Mb in public SNP databases. To fulfill the task of collecting SNP-related data, prioritizing them, and designing genotyping experiments for hundreds or thousands of SNPs, heavy reliance on bioinformatics tools is required. Unfortunately, allele frequency data are dispersed in several different databases. Also, 31% of the 5 or 3 flanking sequence of the reference SNPs in dbSNP are less than 200 bp. Batch query for SNP sequence and allele frequency data is not intuitively simple with existing tools. SNP sequences mostly are not in the format required by primer design software. Most SNP genotyping methods do not provide primer design software in batch mode. Not all SNPs have map information. And so on. All these problems slow down the progress of SNP-based region-wide association study. A SNP Information Mining Pipeline (SIMP) is under development to provide real-time SNP-related data to serve the purpose of data mining and experimental design. So far, we have implemented these tools: "DNannotator" for mapping SNPs; "Frequency Finder" for collecting SNP allele frequency data; "SNPSequer" for fetching SNP flanking sequence of length sufficient for primer design; "SNP cutter" for PCR-RFLP experiment design; "PrimerNerd" for FP primer design. All these tools are web-based applications, supporting batch process, accepting uniformed inputs and producing both user and database-friendly outputs. They are accessible at <http://sky.bsd.uchicago.edu>. More components of this pipeline will be implemented in the coming year. SIMP aims to provide a one stop shopping site for informatics needs of SNP data mining for region-wide association study.

Program Nr: 1471 from 2003 ASHG Annual Meeting

**Shrinkage and Likelihood Estimates of Differential Gene Expression in Oligonucleotide Arrays.** *J. Hu, F. Wright.*  
Biostatistics, UNC-CHAPEL HILL, CHAPEL HILL, NC.

The identification of the genes that are most differentially expressed in two-sample microarray experiments remains a difficult problem when the number of arrays is very small. We discuss the implications of using ordinary t statistics and examine other commonly used variants. For multi-probe oligonucleotide arrays, we introduce a simple model relating the mean and variance of expression, possibly with random gene-specific effects. The model is used to obtain maximum likelihood estimates for the degree of differential expression. Like related approaches developed for cDNA arrays, the estimates have natural shrinkage properties that dispense with the need for ad hoc approaches to guard against inappropriately small variance estimates. The likelihood approach enables a comparison to the Cramer-Rao lower bound, and it is shown that the estimates are highly efficient, even for small sample sizes. We demonstrate that our approach performs well compared to other proposed approaches in terms of the false discovery rate. We also show that it performs well in terms of a new criterion based on the area under the receiver-operator characteristic function, in which the degree of differential expression is compared among rejected genes vs. non-rejected genes. The likelihood framework suggests straightforward extensions to comparisons of multiple groups, and perhaps further shrinkage steps applied to the likelihood estimates.

**Identification of active ID element loci in the rodent genome.** *G.L. Odom, J. Robicheaux, P.L. Deininger.* Tulane Cancer Center, Tulane University, New Orleans, LA.

Throughout evolution, the eukaryotic genome has been bombarded by mobile elements. These elements constitute approximately 45% of the human genome. SINEs (short insertional nuclear elements) are the most abundant mammalian mobile elements. SINEs are also considered to be non-autonomous, in that they do not have a coding capacity, and therefore are thought to parasitize the retrotransposition machinery from other elements (namely L1) or cellular functions. SINEs are recognized as a familiar agent of human disease, and must also have had a significant influence in shaping the eukaryotic genome. In humans, Alu elements make up about 11% of the mass of the genome. We estimate conservatively that there is about one Alu insertion in every 200 humans born. These insertions cause ~0.1% of all human germ-line disease and could possibly contribute to somatic cell damage as well. Our lab has reported that recent (disease causing) Alu insertions consistently possess a homopolymeric adenine region (HAR) of >40 residues in length. We hypothesize that the length of the HAR is critical to amplification events of SINEs. Thus identification of SINEs with the longest A-tails would allow the identification of the currently most active SINEs. We have conducted searches of the rodent genome for HARs of 50 adenine residues followed by analysis by Repeatmasker. Following the establishment of Genbank-derived copy numbers of individual ID elements, we discovered two unique subfamilies contributing ~500 copies in the genome, both of which are located on Chromosome 7 of the Rat. We have also demonstrated experimentally by PCR that these copies are recently inserted in the majority of samples tested in *R. norvegicus* compared to that of *R. rattus*. This suggests that these elements have amplified within the past 1 million years, since the divergence of these two rat species. This approach can be used on any sequenced genome to identify the SINE subfamilies most likely to contribute to disease, or to provide population-specific markers.

**Improved Quantitation of Affymetrix GeneChip Microarray Data.** *B. Merriman, Z. Chen, S. Nelson.* Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA.

The Affymetrix GeneChip is a commercially available DNA microarray system for large scale gene expression analysis in human and other organisms. The platform is based on synthesizing short oligo DNA probes (25-mers) at high density (500,000 distinct probes on 1 cm. square glass substrate). In this system, each gene is monitored by a set of 25-mer oligo probes---typically 22 probes per gene, with 11 sequences matching the transcript, and the other 11 obtained by introducing a single base change into these sequences. Their analysis software reduces these 11 hybridization signals per gene into a single measurement of gene expression level, which they call the "Signal". There is little published data that validates this approach to producing a single gene expression number from the 22 measurements available. Moreover, in typical mammalian experiments, it can be seen that the oligo probes within an expressed gene are highly variable in how well they "work", i.e. in their binding affinities and specificities. It is not unusual to see the match probe intensities vary by a factor of 10 within a gene, or to see a mismatch probe give more signal than the corresponding match probe. This suggests that there may be more accurate ways of extracting gene expression information from the underlying oligo hybridization signals provided by the Affymetrix System. We investigate this possibility in detail, based on the analysis of over 2000 human GeneChip experiments. We show a variety of performance statistics for the oligo probes, and present several ways of obtaining more consistent and accurate expression measurements. We compare our alternative quantitation methods with others available, and perform over 100 RT-PCR measurements to resolve discrepancies between methods. Overall, our results suggest that by restricting to a well-behaved subset of the probes on the array, approximately 25% more genes can be detected, and the overall sensitivity and accuracy can be significantly improved. A simple "mask" file is available that can be used to implement our approach within other quantitation schemes.

**Genetic and Transcriptional Analysis of Metabolic Networks.** *M.M. Xiong, J.Y. Zhao.* Human Gen Ctr/Houston HSC, Univ Texas, Houston, TX.

Modern molecular biology is a mainly descriptive and qualitative science. It focuses on studying individual components such as genes and proteins and on the individual interactions between these molecules. It is now recognized that cells phenotypes are determined by complex biological systems, which consist of various networks such as metabolic networks, genetic networks and protein networks, and their different level of organization. Understanding processes of translation of genetic information into gene transcription and protein production, which in turn regulate metabolic networks, is essential for unraveling principle underlying biological processes. Developing hierarchical mathematical models for joint genetic, proteomic and metabolic analysis of cell function, and integrating various types of biological data such as DNA sequences, gene and protein expressions, and metabolic profiles, is a useful tool for achieving this goal. In this report, we first develop a linear model for prediction of concentrations of enzymes and regulatory proteins from DNA sequences. Then, we express the rate of biochemical reaction as a function of concentrations of enzymes, substrates, products and regulatory proteins. We formulate cellular metabolism as a linear programming in which metabolic flux will be constrained by flux balance equations and the largest possible rates of biochemical reactions. Subgradient and sensitive analysis of linear programming will be used to define price directive and resource directive, which will be used to measure the effect of DNA variation on the metabolic activity at the systems level. Nonsmooth analysis will be used to discover the pattern of control of metabolic networks by DNA, enzymes and substrates and to identify core biochemical reactions for cell function. Finally, examples will be provided to illustrate the proposed models and algorithms for joint genetic, transcriptional and proteomic analysis of metabolic networks.

**Haplotype Block Partitioning and Tag SNP Selection Using Genotype Data.** *K. Zhang<sup>1</sup>, Z.S. Qin<sup>2</sup>, J.S. Liu<sup>2</sup>, T. Chen<sup>1</sup>, M.S. Waterman<sup>1</sup>, F. Sun<sup>1</sup>.* 1) Molecular and Computational Biology Program, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089; 2) Department of Statistics, Harvard University, Cambridge, MA 02138.

Recent studies have revealed that the human genome can be divided into discrete blocks of limited haplotype diversity. A small fraction of SNPs (tag SNPs) are sufficient to capture most of the haplotype structure of the human genome. Several methods have been developed to identify the boundary of blocks and corresponding tag SNPs based on known haplotypes. Unfortunately, phase information could be difficult to obtain from diploid samples. In this paper, we develop a method to partition haplotypes into blocks and to identify tag SNPs based on genotype data by combining the dynamic programming algorithms for haplotype block partition and tag SNP selection based on haplotype data with a variation of expectation maximization (EM) algorithm for haplotype inference. The program is successfully tested on a real data set. We assess the effect of both haplotype and genotype data in haplotype block identification and tag SNP selection as a function of a number of factors, including sample size, density or number of SNPs studied, allele frequencies, fraction of missing data and genotyping error rate, using extensive simulations. We find that a modest number of haplotype or genotype samples will result in consistent block partitions. This study provides practical guidelines for designing experiments to identify haplotype block structure and corresponding tag SNPs.

**Neocentromeres in 15q24-26 map to duplicons which flanked an ancestral centromere in 15q25.** *M. Ventura<sup>1</sup>, J.M. Mudge<sup>2</sup>, V. Palumbo<sup>1</sup>, S. Burn<sup>2</sup>, E. Blennow<sup>3</sup>, M. Pierluigi<sup>4</sup>, R. Giorda<sup>5</sup>, O. Zuffardi<sup>6</sup>, N. Archidiacono<sup>1</sup>, M.S. Jackson<sup>2</sup>, M. Rocchi<sup>1</sup>.* 1) Dept DAPEG Sez. Genetica, University of Bari, Bari, Italy; 2) The institute of Human genetics, University of Newcastle, Newcastle upon Tyne, United Kingdom; 3) Dept of Clinical genetics, Karolinska Hospital, Stockholm, Sweden; 4) Centro di Genetica, Ospedale Galliera, Genova, Italy; 5) Istituto di Ricovero e Cura a Carattere Scientifico Eugenio Medea, Lecco, Italy; 6) Dept. Patolg. Umana ed Ereditaria, Sezione di Biologia Generale, Pavia, Italy.

The existence of latent centromeres has been proposed as a possible explanation for the ectopic emergence of neocentromeres in man. This hypothesis predicts an association between the position of neocentromeres and the position of ancient centromeres inactivated during karyotypic evolution. Human chromosomal region 15q24-26 is one of several hotspots and harbours a high density of chromosome specific duplicons, rearrangements of which have been implicated as a susceptibility factor for panic and phobic disorders with joint laxity. We have investigated the evolutionary history of this region in primates and found that it contains the site of an ancestral centromere which became inactivated about 25 million year ago. This inactivation has followed a noncentromeric chromosomal fission of an ancestral chromosome which gave rise to phylogenetic chromosomes XIV and XV in human and great apes. Detailed mapping of the ancient centromere and two neocentromeres in 15q24-26 has established that the neocentromere domains map approximately 8 Mb proximal and 1.5Mb distal of the ancestral centromeric region, but that all three map within 500kb of duplicons, copies of which flank the centromere in Old World Monkey species. This suggests that the association between neocentromere and ancestral centromere position on this chromosome may be due to the persistence of recombinogenic duplications accrued within the ancient pericentromere, rather than the retention of centromere competent sequences per se. The high frequency of neocentromere emergence in the 15q24-26 region and the high density of clinically important duplicons are understandable in the light of the evolutionary history of this region.

Program Nr: 1477 from 2003 ASHG Annual Meeting

**Development and validation of a predictive model for robust primer design.** *J. Sorenson, S. Glanowski, N. Edwards, Y. Wang, C. Fosler, A. Parker, L. Johnston-Dow, R. Fang, M. Furtado, L.-Z. Pham.* Applied Biosystems, 850 Lincoln Centre Dr, Foster City, CA 94404.

The proper design of PCR primers which will consistently amplify well with high specificity under a desired set of PCR conditions is a challenging task and one which often results in much duplicated effort across laboratories. As part of the Applied Biosystems Genome Initiative, scientists at Celera Genomics resequenced the coding and regulatory regions of 23,000 genes in 40 DNA samples. More than 200,000 primers were designed and 18 million sequence reads were generated. From this unprecedented large-scale resequencing project and with this enormous data set, we have learned a great deal about primer design. To address the complexity of primer design, we have developed a predictive model of amplicon quality which takes into account features known to influence successful amplification and sequencing and predicts an *in silico* probability of amplicon success. This model has been formulated and verified using amplification and sequencing results from the Applied Biosystems resequencing project and the Applied Biosystems Resequencing Primer Sets project. The model allows the successful *in silico* discrimination of amplicons which are likely to succeed under the chosen PCR and sequencing conditions versus amplicons which would likely be more successful under alternative chemistry protocols or amplicons which are highly unlikely to succeed under any conditions. The availability of such a model facilitates the robust design of resequencing primers for genes and regions of interest in human and many other genomes.

Program Nr: 1478 from 2003 ASHG Annual Meeting

**An HMM model for scoring peptides against tandem mass spectrometry data.** *Y. Wan, T. Chen.* Dept of Math, University of Southern California, Los Angeles, CA.

Accurate scoring functions are crucial to database search methods for peptide identification via tandem mass spectrometry. Many mathematical models have been proposed to score peptides against mass spectra, but most scoring functions such as Sequest and Mascot do not consider the continuous and complementary information for b and y ions and intensity information of mass peaks in the data. Here we propose a validation method to compare different scoring functions. We obtain 4070 spectra and peptides with significant p-values, and use these as training data. We build an HMM model to capture the continuous and complementary information of ions, and to combine the mass peak intensities and machine errors into emission probabilities. The result of our method is better than previously existing methods.

**Gene expression profiles define mitochondrial pathology in yeast mutants and human disease.** *H. Smeets<sup>1</sup>, L. Eijssen<sup>1</sup>, R. Mineri<sup>2</sup>, R. van Eijsden<sup>1</sup>, C. van den Burg<sup>1,3</sup>, R. Peeters<sup>4</sup>, I. De Coo<sup>5</sup>, H. Scholte<sup>5</sup>, E. Rubio<sup>3</sup>, M. Zeviani<sup>2</sup>, R. Vlietinck<sup>1</sup>, V. Tiranti<sup>2</sup>.* 1) Dept Genetics & Cell Biol, Univ Maastricht, Maastricht, Netherlands; 2) Div Molec Neurogen, Nat Neurolog Inst "C.Besta", Milano, Italy; 3) Dept Pediatr, Academ Hosp Maastricht, Maastricht, Netherlands; 4) Dept Mathematics, Univ Maastricht, Maastricht, The Netherlands; 5) Depts Neurol & Biochem, Erasmus Medical Centre, Rotterdam, Netherlands.

The applicability of mathematical techniques for microarray data analysis and the incorporation of background knowledge into this analysis was tested for the mitochondrial system in yeast and human mutants. Mathematical techniques were derived from basic statistics, clustering, discriminant analysis and principal component analysis (PCA). Background knowledge was incorporated for the yeast data by downloading website ontology annotations automatically. As test data we used the set from Hughes et al. (Cell 102: 109-126 (2000) or [www.rii.com](http://www.rii.com)) containing whole genome (6,000 genes) expression profiles of 300 (mostly) gene knockout experiments in yeast. PCA reduced the dimension of the data set significantly without losing much of the information and reduced noise as well. By subsequent discriminant analysis, we could distinguish mitochondrial gene knockouts from non-mitochondrial gene knockouts (cytoskeleton, cell membrane, cell wall, cytoplasm, lysosome/vacuole/ peroxisome). As a next step, we applied the same approach on Affymetrix GeneCHIP data (U133A) of a group of 18 fibroblast cell lines of patients with Leigh disease, caused by a Surf1-mutation and 11 controls. The Surf1-patients could be reliably distinguished from the controls by a group of 13 mainly cell structure and ribosomal genes. Untested samples, which were analysed next, were correctly classified by this system. It is not clear at this point if these classifiers reflect Surf1-specific pathology or are characteristic for mitochondrial pathology in general. Gene expression studies in patients with mtDNA caused Leigh (8 fibroblast lines with the T8993G/C or T9176C mutation) or other syndromes and patients with secondary mitochondrial pathology are currently being performed to solve this question.

**Common chromosomal inversions and functional genomic variability.** *M. Riel-Mehan*<sup>1</sup>, *M. Almonte*<sup>1</sup>, *N.P. Rao*<sup>2</sup>, *N.B. Freimer*<sup>1,3</sup>, *R.A. Ophoff*<sup>1,3</sup>. 1) Center for Neurobehavioral Genetics, UCLA, Los Angeles, CA; 2) Department of Pathology, UCLA, Los Angeles, CA; 3) Department of Human Genetics, UCLA, Los Angeles, CA.

Recent studies have revealed large blocks of almost identical sequences in particular chromosomal regions, comprising about 5% of the human genome. Non-allelic homologous recombination between these paralogous sequences results in changes of genomic structure, *e.g.* inversions and duplications. Variation in chromosomal architecture is more widespread in the human genome than previously thought. Examples of common inversion polymorphisms are identified on chromosome 8p23 and 4p16 (Giglio 2001; 2002). Duplicated segments spanning up to 300 Kb in length flank both of these regions. The paralogous sequences on chromosome 8p23 are 200 Kb and 300 Kb in length, 4.5 Mb apart, and are 99% identical. The other known pair of segmental duplications on chromosome 4p16 is each 200 kb, spaced 5 Mb apart, and are 94% identical. Each of the segmental duplications of these pairs is in opposite orientation to one another, which is an integral characteristic to their predisposition to inversions. Intra- chromosomal comparison of these sequences revealed that they are 92% identical to each other. This led us to design a probe to see if any other segmental duplications of similar structure existed in the human genome. A BLAST of the human genome with this probe exposed two more occurrences of paired segmental duplications sharing the same sequence and structure as those on chromosomes 8 and 4, as well as a number of lone instances. We are currently performing FISH tests in different ethnic groups to examine these regions for the presence of common inversions. Based on these results, we have developed an algorithm to expand this approach to the entire genome to discover more sets of paired segmental duplications that could predispose to paracentric inversions, and classify them by family. For our study we focus on inverted pairs of segmental duplications between 500 Kb and 10 Mb apart, with a minimum identity of 90%. Identification of these regions will lead to a better understanding of the architecture of the human genome and a possible functional role in human genetic variation.

**Advancement of Electronic PCR.** *P.S. White*<sup>1,2</sup>, *K. Murphy*<sup>1</sup>, *T. Raj*<sup>1</sup>, *R.S. Winters*<sup>1</sup>. 1) Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Pediatrics, University of Pennsylvania, Philadelphia, PA.

We have adapted the originally described electronic PCR algorithm (Schuler, 1997) to perform string searches more accurately and much more rapidly than previously possible. Our implementation (e-PCR2) runs sufficiently fast to allow even desktop machines to quickly query large genomes with very large genomic element sets. In addition, e-PCR2 is multithreaded, interprets all IUPAC nucleotide symbols, allows searches with elements specified by long sequences (such as SNPs), accepts ranges in the expected PCR size input field, requires substantially less memory for analysis of large sequences, and corrects a number of minor flaws causing misreporting of hits in exceptional cases. In comparison tests using a variety of platforms and parameter settings, e-PCR2 consistently performed several hundred times faster than e-PCR under identical conditions for large genomic sequences. Analysis of word size parameter changes determined that a word size of 11 (currently possible only with e-PCR2) was optimal, and running e-PCR2 under these conditions yielded an additional 3-fold increase in speed. Using a search margin of 1,000 bases and a word size of 11, e-PCR2 running on a single 750 Mhz RS64 IV CPU (IBM p660) was able to identify sequence positions for 130,650 STSs in the entire human genome in 53.1 minutes. A similar single-processor-based search for 3.5 million STSs, which approximates the number of non-redundant human SNPs currently in dbSNP, took 277.2 minutes. Using a margin of 50 increased performance 263% at the cost of 2.4% fewer localizations, and using 8 threads further increased elapsed (but not actual) performance 320%. Thus, e-PCR2 provides increased annotation capabilities for complex genomes to non-expert laboratories. e-PCR2 has been compiled for Linux, Solaris, AIX, Windows, and Macintosh platforms. Source and executable code is freely available to the research community at <http://genome.chop.edu/mePCR/>.

Program Nr: 1482 from 2003 ASHG Annual Meeting

**DNA Banking Study in an Ethnically Diverse Urban University Hospital.** *W.A. Wolf, M.J. Doyle, S.A. Aufox, T.M. Frezzo, M.E. Smith, W.A. Kibbe, R.L. Chisholm.* Center for Genetic Medicine, Northwestern University Chicago, IL.

The Northwestern University gene bank (NUgene) is a genetic banking project with a centralized genomic DNA sample collection and storage system that anonymizes DNA samples and regularly updates phenotypic data from electronic medical records (eMR). The purpose of NUgene is to provide an ethnically and medically diverse population of samples that will allow association of specific genetic variants with disease and therapeutic outcomes. NUgene's DNA samples and associated phenotypic information are available to internal and external researchers with Northwestern University IRB approved studies. We plan to enroll 100,000 participants over 6 years. Eligibility criteria includes the ability to provide informed consent, persons who are 18 years or older and who receive medical care through Northwestern Memorial Hospital or one of its affiliates. Participants are recruited through a variety of clinical settings and physician groups. Consenting individuals complete a self-administered family and medical history questionnaire, a consent form and a HIPAA authorization form in a single encounter. Phenotypic and genotypic data are housed in a separate database from participant identifiers and enrollment data to enhance security. Phenotypic information is updated biannually from eMR. To make gene-disease associations, we will use the Disease Ontology to link known human disease information to our genotype/phenotype repository. Recruitment began in November 2002 and over 400 participants are currently enrolled. Across venues, enrollment uptake is 10% of those approached to participate. The majority (77%) of participants come from a general internal medicine clinic. Participants represent the diverse nature of the Chicago community, with representation from 9 major ethnic groups. Females are slightly overrepresented in our sample at 62% and ages range between 19 and 85. 70% of our participants have a college or graduate degree. The NUgene project is jointly supported by Northwestern University, Northwestern Memorial Hospital and Evanston Northwestern Healthcare.

**Phenotype in locus-specific databases: contents and directions.** *S.A. Teebi<sup>1</sup>, R.G.H. Cotton<sup>2</sup>*. 1) Centre for Computational Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) Genomic Disorders Research Centre, St. Vincent's Hospital, Melbourne, Australia.

Discussions of phenotype content in locus-specific mutation databases (LSDBs) have until now been vague and under-informed. Specifically, these databases, which in turn largely dictate the contents of whole genome variation databases like HGVbase and dbSNP, have, by and large, been assumed to be vacant of significant clinical and phenotype-related information. Here we present the results of a study conducted with the aim of ameliorating the extant knowledge on this field and using the new knowledge to outline possible directions to be taken in the development of phenotype-conscious databases in the future. 134 LSDBs listed by the HGVS (<http://www.hgvs.org>) and MutRes (<http://srs.ebi.ac.uk/srs6bin/cgi-bin/wgetz?-page+LibInfo+-newId+-lib+MUTRES>) resources were polled, covering 282 genes. 82% of LSDBs were found to record some or any phenotype information. 377 total phenotype-related fields were found in all the databases, an average of 3.53 fields per database. A significant percentage of fields contained just one phenotype field (30%). A low percentage of LSDBs had in excess of 10 phenotype fields (3%). In general, phenotype parameters in LSDBs exhibited a great deal of heterogeneity (i.e. different data were recorded for different loci) and sparseness (i.e. phenotype data were not often present in every mutation entry in a given database). Other findings indicated a great level of willingness among LSDB curators to record phenotype, as well as an understanding of what needs recording. Despite this, a naivety in regards to classifying and tabulating the difficult parameter of phenotype was evinced. To tackle this "phenotype problem", especially as it pertains to whole-genome databases and their interoperability with LSDBs, we propose a method for the recording of such data that is based on the Entity-Attribute-Value (EAV) model. Basic aspects of this model will be discussed.

Program Nr: 1484 from 2003 ASHG Annual Meeting

**A tool for "Genomic Convergence": Annotating the Ensembl human genome browser with disease-specific graphical representations of linkage analysis data.** *J.E. Stenger, H. Xu, C. Haynes, R. Cornwell, E. Hauser.* Center for Human Genetics, Institute for Genome Science and Policy, Duke University, Durham, NC.

"Genomic Convergence" is a multi-step data reduction strategy that merges the resulting potential candidate gene lists obtained by different laboratory and statistical approaches to prioritize candidate disease susceptibility genes that may be contributing to complex human diseases [Hauser et al., 2003] We are using this approach at the Duke Center for Human Genetics to identify candidate susceptibility genes that warrant subsequent association analysis by identifying the subset of genes that are within regions with high LOD scores greater than one and are also identified by either serial analysis of gene expression (SAGE) analysis or gene chip technology as having a significant alteration in the levels of mRNA expression. For this purpose we have exploited the Ensembl open source genome annotation system and database ([www.ensembl.org](http://www.ensembl.org)) which we maintain an instance of on our local servers in conjunction with the Distributed Annotation System (DAS) [Dowell, Eddy & Stein] to store our laboratory-specific data on our local secure server and to visually display this data in the context of the Ensembl genomes browser We also have previously developed high-throughput bioinformatics methods to map the location of SAGE tags and probes spotted on the Affymetrix gene chip sets (which we have contributed to Ensembl.org) on to the human genome working draft sequence builds. To facilitate genomic convergence, we have created a database on our DAS server to hold coordinates from both single point and multipoint linkage analysis pertaining to different research projects and have developed an algorithm that uses the stored coordinates to draw a graph, selected by the user from a pull down box, plotting LOD scores on the y-axis vs.the position in base pairs on the X-axis. This graph is displayed under the ideogram of the chromosome that is in view. This graph maps markers from the genetic position on Decode map to their position in base pairs and seamlessly integrates into the Ensembl display environment.

**Adding annotation to identify at-risk DNA motifs in unknown Genes Identify.** *T. Wang, J.E. Stenger.* Center for Human Genetics, Duke University Medical Center, Durham, NC.

Following a genomic scan, the research team identifies a region defined by two peak LOD scores. A bioinformatics savvy geneticist then brings up the human genome browser page at [ensembl.org](http://ensembl.org), and clicks on export data. From there she downloads all the candidate genes between the two markers denoted by the peaks. Of the list of 55 candidate genes, only 21 have the function associated with them. The geneticist, not knowing what to do with the genes without a function, focuses entirely on the genes with a function. This very real scenario drives home the message that without more annotation many important genes may be overlooked. The first thing we can do is analyze the human genome for sequence motifs that laboratory research has indicated are primed for instability. Our previous research has shown that closely-spaced, highly identical, inverted Alu pairs with homologous regions > 250 base pairs are likely to undergo homologous recombination in yeast model systems (Lobechev et al., 2000). Using computational methods we have been able to determine the Alu pairs in the human genome working draft sequence that meet this set of criteria (Stenger et al., 2001). By identifying such biases we have a clue as to what is not well-tolerated in the genome. This gives us the ability to predict which genes are likely to have their functions abrogated by undergoing a homologous recombination event. DNA repeats, be they microsatellites, or inverted, highly identical, short distant repeats (SINES) and long interspersed repeats (LINES) are ubiquitous in all genomes. These repeats, under certain mitigating circumstances, such as in the presence of the mutator phenotype (mismatch repair defective) or helicase defects, are length dependent fragile sites in yeast and higher eukaryotes. Collectively, these DNA motifs have been coined as at-risk factors for genomic alterations by Gordenin and Resnick (1998). Genes containing at-risk motifs have a high likelihood of contributing to disease. Annotating the genome with such at-risk motifs is therefore a reasonable thing to do to help identify genes associated with complex disease.

**Microarray gene expression analyses of multiple sclerosis plaque tissue.** *L.R. Griffiths<sup>1</sup>, L. Tajouri<sup>1</sup>, A.S. Mellick<sup>1</sup>, E.G. Tannenber<sup>2</sup>, W.W. Tourtellotte<sup>3</sup>.* 1) Genomics Research Centre, Griffith University, Parklands Drive, Southport QLD Australia 4215; 2) QLD Medical Laboratories, West End QLD Australia; 3) Human Brain & Spinal Fluid Resource Center, Wilshire Blvd, LA. Cal. USA.

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system, characterized by zones of demyelination and inflammatory plaques. Symptoms include limb weakness, sensory loss, visual alterations and bladder dysfunction, and the appearance of lesions or plaques that are disseminated in time and space. In this study, we have employed array hybridisation and Q-PCR analysis to examine gene expression changes, that may differentiate inflammatory forms of MS plaques. RNA from MS chronic active and MS acute lesions was extracted, and compared with patient matched normal white matter using fluorescent cDNA microarray hybridisation analysis. This resulted in the identification of 139 genes that were differentially regulated in MS plaque tissue compared to normal tissue. Of these, 70 transcripts were uniquely differentially expressed (>1.5-fold) in either acute or chronic active tissues, while 69 genes showed a common pattern of expression in the chronic active and acute plaque tissues investigated ( $P < 0.0001$ ,  $r = 0.73$ , by Spearman's analysis). For these 69 genes, the mean fold level of gene expression difference was found to be significantly higher in acute plaques, suggesting that symptomatic variation in inflammatory lesions in MS may be the result of overall quantitative, rather than specific gross qualitative differences in gene activity. In this study, SYBR Green I, real time Q-PCR was also used to validate the differential expression of array selected clones and to examine the expression of transcripts that may be present below the level of sensitivity for array analysis. The determinations of fold regulation, obtained by Q-PCR analysis of 7 array-selected genes, were found to correlate significantly with determinations of fold obtained following fluorescent array analysis, of chronic active and acute plaque tissue ( $r = 0.75$ ,  $P < 0.01$ , by Pearson's bivariate correlation,  $n = 14$ ).

**Evidence for a hominoid specific splicing form of neuropsin, a gene involved in learning and memory.** *Y. Li<sup>1</sup>, B. Su<sup>1,2</sup>*. 1) Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming, Yunnan, China; 2) Center for Genome Information, Department of Environmental Health, University of Cincinnati, Cincinnati, OH.

Neuropsin is a secreted-type serine protease expressed in the brain and involved in learning and memory. We sequenced exon 3 and its flanking introns of neuropsin gene containing the alternative splicing site in humans and representative nonhuman primate species. We found that the Type-II splicing form of neuropsin is specific to hominoid species (humans and apes), indicating that this splicing form originated about 18 million years ago. The synonymous and nonsynonymous mutation analysis revealed the signature of adaptive evolution of Type II neuropsin due to Darwinian positive selection. Hence, this hominoid specific splicing form of neuropsin may contribute to the progressive changes of cognitive skills during primate evolution.

**Rapid evolution of microcephalin, the gene determining human brain size.** *YQ. Wang<sup>1</sup>, B. Su<sup>1,2</sup>*. 1) Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming, Yunnan, China; 2) Center for Genome Information, Department of Environmental Health, University of Cincinnati, Cincinnati, OH.

Microcephalin is a major player in human brain development. Truncated mutations in this gene can cause primary microcephaly in humans with a brain size comparable with that of early hominids. We sequenced two exons and flanking introns of the microcephalin gene in humans and seven nonhuman primate species including great apes, lesser apes and Old World monkeys. Our results showed that there are frequent amino acid substitutions with 23.6% of the sites being variable in humans and nonhuman primates. The nonsynonymous substitution rate ( $K_a$ ) between humans and rhesus monkeys is 0.05, which ranks microcephalin one of the fastest evolving proteins in primates. There are 5 fixed and 8 polymorphic amino acid substitutions in human populations, most of which are conserved in apes and Old World monkeys. The Fu's  $F_s$  neutrality test and individual codon substitution test in human populations are against the neutral expectations, suggesting the accelerated evolution of microcephalin gene in humans is likely the consequence of Darwinian positive selection during human evolution.

**Adaptive Evolution of ASPM During Human Origin.** XJ. Yu<sup>1</sup>, B. Su<sup>1,2</sup>. 1) Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming, Yunnan, China; 2) Center for Genome Information, Department of Environmental Health, University of Cincinnati, Cincinnati, OH.

ASPM (abnormal spindle-like microcephaly associated gene) is essential for normal mitotic spindle function in human embryonic neuroblasts. Mutations of ASPM can cause human autosomal recessive primary microcephaly (MCPH) with a brain size comparable with that of early hominids. Therefore, ASPM may play an important role in brain enlargement during primate evolution, especially human origin. We sequenced a 3700 bp fragment of exon 18 of ASPM in seven representative primate species, including humans, chimpanzees, gorillas, orangutans, gibbons and two Old World monkey species. Our result showed that there are nine fixed sequence substitutions within the coding region of exon 18 in humans, and eight of them are replacement mutations. The calculation of Ka (nonsynonymous mutation rate)/Ks (synonymous mutation rate) revealed an accelerated amino acid substitutions of exon 18 in the human lineage. The Ka/Ks ratios are 1.63 between humans and chimpanzees and 3.52 between the putative human ancestor and modern humans. The McDonald-Kreitmans neutrality test is significant ( $P = 0.047$ ), indicating adaptive evolution, i.e. Darwinian positive selection of ASPM during human origin.

**Analysis of APOE specific hippocampal gene expression in Alzheimers Disease (AD) using Serial Analysis of Gene Expression (SAGE).** *P. Xu<sup>1</sup>, C. Kroner<sup>1</sup>, C. Browning<sup>1</sup>, C. Hulette<sup>2</sup>, D.E. Schmechel<sup>2</sup>, J. Ervin<sup>2</sup>, M. Hauser<sup>1</sup>, M.A. Pericak-Vance<sup>1</sup>, J.R. Gilbert<sup>1</sup>.* 1) Center for Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Alzheimer Disease Research Center, Duke Univ Medical Ctr, Durham, NC.

Serial analysis of gene expression (SAGE) is a powerful technique for the generation of quantitative information about tissue specific gene expression. SAGE can detect small changes in expression level and transcripts from entirely novel genes. To identify new genes conferring susceptibility to Alzheimers disease (AD), and study the influence of APOE genotype on gene expression in AD, we have constructed four SAGE libraries: one each from APOE3/3, APOE3/4 and APOE4/4 Alzheimers patients, and one from APOE3/3 control samples, using total RNA isolated from the hippocampus of AD patients and normal controls. Initial analysis of APOE 3/3 AD and control SAGE libraries showed each contained more than 400,000+ tags (16,000 ~ 19,000 clones). Preliminary sequence results confirm that the PCR amplified DNA fragments contain concatemers composed of an average 26~28 bp ditags. We have picked, cultured and commenced sequencing using an ABI 3700 automated sequencer more than 3,000 clones from each APOE 3/3 library to generate initial data from a minimum of 60,000 tags. Sequence analysis of APOE 3/4 and 4/4 libraries will commence once this initial sequencing is finished. Sequence files are analyzed by means of the eSAGE software, which identifies the anchoring enzyme site with proper spacing and extract the two intervening tags and records them in a database. We will evaluate gene expression patterns in AD and normal control SAGE libraries by comparing the abundance of individual tags, and identifying the corresponding genes using the tag-to-gene mapping database generated from NCBI's UniGene sets. Through the combined use of both Affymetrix microarrays and SAGE, we will obtain a detailed and quantitative picture of known and novel genes expressed in the normal and AD hippocampus.

**Integration of CGH data and microarray gene expression data aids in the discovery of DNA-based diagnostic tumor markers.** *C.C. Harris*. Exagen Corporation, Albuquerque, NM.

The maturation of microarray technology has enabled the routine collection of genome-wide gene expression (RNA) data. In cancer diagnostics, several authors have shown that microarray data collected from tumors may be useful in differential diagnosis, tumor staging and prognosis. The data produced by these studies ideally represents a valuable resource for the development of new diagnostics. However, at present, the application of microarray technology requires steps in sample collection and sample preparation that inhibit routine clinical adoption.

In contrast, DNA-based markers are commonly used in cancer diagnostics. Diagnostic implementations utilizing FISH and RT-PCR technology are in widespread use. New diagnostic products based on such accepted technology will more quickly find clinical acceptance. A major obstacle to the development of new DNA-based diagnostics is the lack of genome-wide gene-scale DNA data. However low-resolution band scale DNA copy number information is available in the form of comparative genomic hybridization (CGH) data. While clinical application of the CGH process for diagnostic purposes is limited by the technical difficulties associated with the process, this does not preclude the use of existing CGH data in the identification of new diagnostic markers. We propose that the integration of band-scale CGH data and gene-scale microarray gene expression data is a viable path to the development of new clinically relevant and clinically useable DNA-based cancer diagnostics.

As a demonstration, we have analyzed publicly available microarray and CGH data collected from glioblastoma and anaplastic oligodendroglioma tissue samples. Notably, the analysis of each dataset independently did not result in the identification of statistically significant markers discriminative of phenotype. Concurrent analysis, by way of identifying gene-specific markers consistent across both datasets, resulted in statistically significant markers ( $p < .05$ ). Markers identified in such a manner can be easily implemented for validation studies and ultimately as diagnostic products.

**Gene Expression in Congenital Heart Disease.** *D. Bittel, N. Kibiryeve, G.K. Lofland, J.E. OBrien, M.G. Butler.*  
Childrens Mercy Hospitals and Clinics and University of Missouri-Kansas City School of Medicine, Kansas City, MO.

Congenital heart disease occurs in 0.5 to 1% of all newborns and represents a significant proportion of birth defects. Due to the high number of infants with conotruncal defects treated at Childrens Mercy Hospital compared with other pediatric centers, the Sections of Cardiovascular & Thoracic Surgery and Medical Genetics have established a program to elucidate the underlying epidemiological, physiological and molecular genetic mechanisms responsible. Most congenital heart disease is thought to be multifactorial in origin implying the involvement of anomalous gene expression and epigenetic factors. Recent advances in microarray technology, allowing for the simultaneous study of novel genome-wide expression of human genes, are aiding the search for the genetic causation of conotruncal defects. We have examined gene expression in dysplastic pulmonic valve from two nonsyndromic infants (a 7 month old female and a 6 month old male) with tetralogy of Fallot (TOF) and comparable tissue from a control infant (1 month old male) with no heart disease. We have examined in excess of 12,800 genes using the Agilent Technologies Human 1 cDNA microarray to identify genes involved in conotruncal defects. Initial comparisons in the affected infants were made between dysplastic pulmonic tissues and pericardium. In those comparisons, more than 450 genes were found to express differently ( $\pm 10X$ ) in the two tissues: Cell cycle or apoptosis factors, 18; Structural proteins, 18; Calcium binding, 2; Transcription, translation or processing factors, 40; Metabolic factors, 29; Growth factors or kinases, 28; Signaling proteins, 41; Homeostasis factors, 25 and Unclassified factors, 38. Subsequent gene expression comparisons with pulmonary artery from our control infant focused on known candidate genes for cardiac defects. JAG1 was detectable in the control infant but had no detectable expression in the affected infants by microarray analysis or quantitative RT-PCR. This was confirmed in three additional infants with TOF. JAG1 is a promising candidate gene for isolated TOF and is known to be causative for Alagille syndrome in which cardiac defects are described.

**Regulation of Metabolism Protein, HPRT in CD8+ Cells of Human Immunodeficiency Virus Type-1 Infected Subjects on Highly Active Antiretroviral Therapy.** *E.M. McGhee, L.S. Diaz, H.G. Foster.* University of California San Francisco, Department of Community Health Systems, UCSF Comprehensive Cancer Center, Medicine.

Previous studies have shown that genetic changes observed in human immunodeficiency virus type-1 (HIV-1) infected peripheral blood monocyctic cells in the era of highly active antiretroviral therapy (HAART) may be involved in abnormal purine metabolism-hyp oxanthine-guanine-phosphoribosyl transferase (HPRT) regulation, genetic polymorphisms, and increased mutation frequency. HPRT is an enzyme that catalyzes the transfer of the phosphoriboyl moiety of 5-phosphoribosyl-1-pyro-phosphate to the 9 position of hypoxanthine and guanine to form inosine monophosphate and guanosine monophosphate. Also, in instances of mutational damage in the HPRT gene, cells may commit to an increased sequence analysis of mutations indicating the formation of single-base, transversion (G -> T) and transition (G -> A) substitutions. Overall, this indicates that patients infected with HIV-1 and exposed to nucleoside analog and protease inhibitor drugs, may sustain significant genotoxic insults and should be therefore subjected to long-term surveillance. Given these evidences for genetic damage in these patients, consideration should be given to HAART, and HAART induced-long term genomic instability. We are currently investigating genomic damage at the HPRT locus in HIV-1 infected cells from subjects receiving HAART. We show that HPRT is down regulated in CD8+ cells from subjects receiving HAART. cDNA from CD8+ cells of HIV-1 infected subjects receiving HAART, those no longer on HAART, and uninfected people were analyzed by microarray analysis to determine gene expression of the HPRT gene. Microarray analysis for gene expression indicated that HPRT is down regulated in CD8+ cells from HIV-1 infected subjects on HAART when compared to people that are uninfected, and subjects infected but not on HAART. Down regulation of the HPRT gene in cells from HIV-1 infected subjects on HAART, may impose limitations for effective optimal treatment in HIV-1 patients. Therefore, in a population based study we are analyzing additional CD 8+ cells from subjects receiving HAART, and using RT-PCR to determine mRNA expression of other proteins that may be involved in abnormal purine metabolism. This information can be helpful in advancing our knowledge of protein function in biochemical metabolism in HIV-1 infect subjects in the era of HAART, and could lead to new strategies for better therapy.

**Dissecting the molecular bases of kidney stone disease by microarray analysis.** *L. Liang<sup>1</sup>, J. Chen<sup>1</sup>, R. Vittal<sup>2</sup>, L. Deng<sup>1</sup>, Z.E. Selvanayagam<sup>3</sup>, J.A. McAteer<sup>4</sup>, J.A. Tischfield<sup>1</sup>, K.V. Chin<sup>2</sup>, A. Sahota<sup>1</sup>.* 1) Dept of Genetics, Rutgers Univ, Piscataway, NJ; 2) Dept of Chemical Biology, Rutgers Univ, Piscataway, NJ; 3) Dept of Pediatrics, UMDNJ, New Brunswick, NJ; 4) Dept of Anatomy and Cell Biology, Indiana University, Indianapolis, IN.

Renal deposition of calcium oxalate monohydrate (COM), primarily due to idiopathic causes, accounts for the vast majority of cases of stone disease. Genetic causes of stone disease are rare, but over 30 disorders have been described including 2,8-dihydroxyadenine (DHA) nephrolithiasis due to adenine phosphoribosyltransferase (APRT) deficiency. The interaction of crystals with renal tubular epithelial cells may be a critical event in the initiation of the disease process. In an earlier cDNA microarray study using a small number (588) of genes, we showed that DHA and COM crystals induced qualitatively similar gene expression changes in primary cultures of human kidney epithelial cells. To verify whether DHA stone disease can be used as a model for the more common COM stone disease, we carried out a detailed microarray study involving over 10,000 transcripts and with cells exposed to crystals for up to 48 h. Expression changes for a selected number of genes were confirmed by Real Time RT-PCR. Both DHA and COM crystals induced time-dependent changes in gene expression, but very few genes were detected at the early exposure stages. Most of the expression changes occurred at the intermediate or late stages, which may reflect the time required for crystals to attach to or be internalized by the epithelial cells. As in our previous study, DHA and COM crystals induced similar patterns of gene expression, but a small number of crystal-specific expression changes were also observed. In particular, DHA crystals, but not COM crystals, induced a significant increase in the expression of a cluster of cytokines that may be involved in the inflammatory process. Inflammation of kidney tissue is rare in COM stone disease, but is common in APRT deficiency. These findings suggest that the cell culture system may be useful for identifying the common events underlying stone disease as well as for differentiating crystal-specific effects.

**Towards high-throughput *RAII* mutation screening by temperature gradient capillary electrophoresis.** *R.E. Slager*<sup>1</sup>, *T.L. Newton*<sup>2</sup>, *E.S. Wilch*<sup>1</sup>, *C.N. Barth*<sup>2</sup>, *A. Rohr*<sup>3</sup>, *B. Finucane*<sup>4</sup>, *P.I. Patel*<sup>3</sup>, *S.H. Elsea*<sup>1,2</sup>. 1) Genetics Program, Michigan State University, East Lansing, MI; 2) Department of Zoology, Michigan State University, East Lansing, MI; 3) Department of Neurology, Baylor College of Medicine, Houston, TX; 4) Elwyn Training and Research Institute, Elwyn, PA.

Smith-Magenis syndrome (SMS) is a multiple congenital anomalies and mental retardation syndrome associated with deletions involving chromosome 17p11.2. Through careful clinical and molecular evaluation, we recently reported the identification of three distinct frameshift mutations in the retinoic acid induced 1 (*RAII*) gene in three unrelated individuals who display the characteristic SMS behavioral and physical phenotype but who do not harbor a detectable deletion on 17p11.2. These mutations, a 29 base pair deletion in SMS129, and deletions of a single C in SMS159 and SMS156, were detected through PCR amplification and sequencing of the coding region and known splice variants of *RAII*. Our pilot sequencing experiments also encompassed three nondeletion patients who displayed several phenotypic aspects of the syndrome but displayed some features discordant with SMS. We did not find any deleterious *RAII* mutations in this sample group. Since *RAII* is a large and complex gene, our current studies have focused on developing an effective high-throughput mutation screening method. Using the SpectruMedix temperature gradient capillary electrophoresis (TGCE) system, we demonstrate that we are able to distinguish the three deletion mutations from known control samples. We also show that TGCE can identify known single nucleotide polymorphisms within *RAII*. The TGCE method is desirable for high-throughput mutation screening because the cost per sample is low, and we are able to use diluted, crude PCR product in the analysis without losing adequate signal. We report the *RAII* mutation screening results of 10 putative SMS nondeletion patients by analysis of PCR products by TGCE followed by sequence confirmation of any potential mutations. We believe that the TGCE method is a reliable and cost-effective method to determine the spectrum of *RAII* mutations in the SMS population.

***Drosophila* model of Down syndrome.** K.T. Chang, Y.J. Shi, K.T. Min. Neurogenetics Branch, NINDS, NIH, Bethesda, MD.

Down syndrome (DS), a disorder that affects 1 in 700 births, is the most common genetic cause of mental retardation and other abnormalities. Despite much effort, the underlying cause of mental retardation in DS remains elusive. Down Syndrome Critical Region 1 (DSCR1) gene, located within the DS critical region on chromosome 21, is overexpressed in the brain of Down syndrome fetus and encodes an inhibitor of calcineurin, but its physiological significance is unknown. An attractive system for studying the functions of DSCR1 is *Drosophila melanogaster*; it has a short generation time, and is easily amenable to genetics, biochemical, molecular, and behavioral analyses. We thus generated *Drosophila* loss-of-function and overexpression mutants of nebula, an ortholog of human DSCR1. Biochemical studies show that the nebula protein can bind to and inhibit calcineurin activity, as well as regulate cAMP-dependent protein kinase activity, CREB phosphorylation, and CREB-dependent gene transcription. Behavioral analyses revealed that the nebula mutant exhibits defective learning and long-term memory. Strikingly, transgenic flies overexpressing nebula also show severely impaired learning. Furthermore, we demonstrate that the same calcineurin-mediated signaling pathway is altered in human trisomy 21 fetal brain tissue overexpressing DSCR1. Together, these results suggest that DSCR1 mediates learning and long-term memory, and that alteration of DSCR1 expression could be one cause of mental retardation in Down syndrome.

**Dysregulation of Interferon inducible and Aquaporin 1 genes in the blood of patients with Sjogrens syndrome.**

*E.S. Emamian, M.E. Grandits, C.M. Meyer, A.N. Leiran, J. Ermer, E.C. Baechler, T.W. Behrens, N.L. Rhodus, K.L. Moser.* Medical School, University of Minnesota, Minneapolis, MN.

Sjogrens syndrome (SS) is an autoimmune disorder characterized by lymphocytic infiltration into lacrimal and salivary glands leading to symptomatic dry eyes and mouth. Disease mechanisms are not well defined but include production of autoantibodies, dysfunction of molecular water transport processes, dysregulation of apoptosis, and alterations in cytokine activity. To gain insight into the underlying etiology of this disease, we have used microarray technology to profile global gene expression of whole blood from SS patients and controls. Initial studies indicated that differential gene expression patterns were detectable in SS patients compared with healthy controls and suggested that numerous genes induced by interferons (IFNs) were overexpressed in patients. We have now enlarged the patient sample (n=33), evaluated a second independent matched control group (n=18), and increased the number of genes interrogated from approximately 12,600 to 22,000. All patients fulfilled the 2002 revised European criteria for classification of SS. Labeled cRNA was generated from the cDNA sample by an in vitro transcription (IVT) reaction and fragmented cRNA was hybridized to Affymetrix Human U133A Microarray Chips. After scanning and scaling gene expression values, the expression levels of transcripts from patients and controls were compared using unpaired students t-test. Over 330 genes were differentially expressed using nominal significance criteria of  $p < 0.001$ , and over 40 of these genes differed in mean expression levels by greater than 1.5 fold. The results confirmed a significant over-expression of genes involved in the control of interferon responses or that are inducible by IFN. Among the down-regulated genes, the most significant was aquaporin1 (AQP1,  $P=0.00058$ ). The down-regulation or dislocation of AQP1 has been considered to have role in dysfunction of the salivary gland in SS. These results implicate a role for IFN-related pathways in the pathophysiology of SS, and identify several candidate genes that may be useful as biomarkers in the diagnosis of this disease.

**The 9q subtelomeric microdeletion syndrome; Evidence for a genomic susceptibility.** A. Huang<sup>1</sup>, D.R. Stewart<sup>1</sup>, F. Faravelli<sup>2</sup>, B.M. Anderlid<sup>3</sup>, M. Kaur<sup>1</sup>, E. Rossi<sup>4</sup>, R. Tenconi<sup>4</sup>, M. Nordenskjöld<sup>3</sup>, K.W. Gripp<sup>5</sup>, W.S. Meschino<sup>6</sup>, O.W.J. Quarrell<sup>7</sup>, M. Irons<sup>8</sup>, C.A. Zaleski<sup>9</sup>, M. Budarf<sup>1</sup>, E.H. Zackai<sup>1</sup>, N.B. Spinner<sup>1</sup>, I.D. Krantz<sup>1</sup>. 1) Div of Hum Genet and Mol Biol, The Children's Hosp of Philadelphia, PA; 2) S.C. Genetica Umana, Ospedale Galliera, Genoa, Italy; 3) Dept of Mol Med, Karolinska Inst, Stockholm, Sweden; 4) Univ di Pavia/Univ di Padova, Italy; 5) AI duPont Hosp for Children, Wilmington, DE; 6) North York Gen Hosp, Dept of Genetics, Toronto, Canada; 7) Sheffield Childrens Hosp, Sheffield, UK; 8) Div of Genet and Metab, Boston Childrens Hosp, MA; 9) Marshfield Clinic, Marshfield, WI.

With the recent advent of clinical testing for subtelomeric rearrangements, several submicroscopic deletions of chromosome 9q have been reported. Cytogenetically visible deletions of this region are rare with only 2 reported cases (Ayyash et al, J Med Genet 34:610; Schimmenti et al, Am J Med Genet 51:140), likely indicating a decreased viability for affected fetuses. The 9q subtelomeric deletion syndrome has a recognizable pattern of malformations including hypertelorism, eyebrow and ear abnormalities, a high arched palate, a tented upper lip, hypotonia, mental retardation, and conotruncal heart defects. Cytogenetic and molecular mapping of the deletion breakpoints in 12 probands using a series of 8 FISH probes, 14 microsatellite markers, and 14 SNPs revealed an apparent common proximal breakpoint in 11/12 probands, similar to what has been seen in other microdeletion syndromes. This breakpoint maps between SNP C12020842 (rs13631) centromerically and SNP C80658 (CACNA1B gene) telomerically and falls within a gap in the published genomic sequence (~1 Mb). The gap falls between clones RP11-350O14 and CTD-2521H7 proximally and RP11-417A4 distally with the proximal clones terminating in the same region, suggesting that it may be unclonable in bacteria. Unclonable human sequences have been associated with regions of the genome that undergo chromosomal rearrangement. Molecular definition and analysis of this region using 10 additional SNPs mapped between the two proximal breakpoint flanking SNPs in these patients is underway.

**Expression atlas of the mouse orthologues of the Williams-Beuren syndrome critical region genes.** *A. Reymond<sup>1</sup>, M. Yaylaoglu<sup>2</sup>, G. Merla<sup>1</sup>, C. Thaller<sup>3</sup>, C. Caccioppoli<sup>4</sup>, C. Ucla<sup>1</sup>, D. Montanaro<sup>4</sup>, T.M. Chen<sup>3</sup>, N. Scamuffa<sup>1</sup>, A. Ballabio<sup>4</sup>, S.E. Antonarakis<sup>1</sup>, G. Eichele<sup>2</sup>, V. Marigo<sup>4</sup>.* 1) Division of Medical Genetics, University of Geneva Medical School; 2) Max Planck Institute, Hannover; 3) Baylor College of Medicine, Houston; 4) TIGEM, Naples.

The Williams-Beuren syndrome (WBS) is a neurodevelopmental disorder characterized by mental retardation with unique cognitive and personality profile, distinctive facial features, supraaortic stenosis, short stature, connective tissue anomalies, hypertension, infantile hypercalcemia, dental and kidney abnormalities and premature aging of the skin. Its incidence is estimated at 1/15,000 and sporadic de novo inheritance is usual. Molecular basis of the syndrome is a heterozygous ~1.5 Mb microdeletion or inversion at chromosome band 7q11.23. While our understanding of the etiology of WBS has improved greatly, we are still ignorant as to the molecular basis of all except the cardiovascular phenotype. To define where the 31 WBS critical region genes exert their function and identify their possible role in the WBS phenotypes we performed a systematic analysis of the expression profile of all their murine orthologues. To obtain an high resolution expression pattern several complementary methods were combined: RT-PCR on a mouse cDNA panel of 12 adult tissues and 4 developmental stages; wholemount in situ of E9.5 and E10.5 embryos, section in situ of E14.5 embryos, brain section in situ of E15.5 embryos, P7 and P56 mouse. These stages correspond to mid and late embryonic and fetal human periods, when the major organs and body regions are organized, while the brain sections allow to correlate expression with neuroanatomical changes described in WBS patients. The topographical catalogue of expression of the murine orthologues of WBS genes will be instrumental to the understanding of the pathogenesis of this contiguous gene syndrome. The entire data set will be comprehensively documented on a freely accessible web page, which will lists the WBS genes, their murine orthologs and the probes used for ISH and displays images as well as annotation tables.

**Comparative genomic hybridization using a proximal 17p BAC/PAC array detects rearrangements responsible for four genomic disorders.** *C.J. Shaw<sup>1</sup>, C.A. Shaw<sup>1</sup>, W. Yu<sup>1</sup>, P. Stankiewicz<sup>1</sup>, L.D. White<sup>1</sup>, A.L. Beaudet<sup>1</sup>, J.R. Lupski<sup>1,2</sup>.* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept Pediatrics, Baylor Col Medicine, Houston, TX.

Proximal chromosome 17p is a region that is rich in low copy repeats (LCRs) and prone to chromosomal rearrangements. Four genomic disorders map within the interval 17p11-p12: Charcot-Marie-Tooth disease type 1A (CMT1A), hereditary neuropathy with liability to pressure palsies (HNPP), Smith-Magenis syndrome (SMS) and dup(17)(p11.2p11.2) syndrome. While 80-90% or greater of the rearrangements resulting in each disorder are recurrent, several non-recurrent deletions/duplications of varying sizes within proximal 17p also have been characterized using fluorescence in situ hybridization (FISH). Here we tested the ability of a BAC/PAC array-based comparative genomic hybridization (array-CGH) method to detect these genomic dosage differences and map breakpoints in 26 patients with recurrent and non-recurrent rearrangements. We found that array-CGH detected the dosage imbalances resulting from either deletion or duplication in all the samples examined. Furthermore, the array-CGH approach mapped 46/47 (97.9%) of the analyzed breakpoints to within one overlapping BAC/PAC clone compared to that determined independently by FISH. In addition, array-CGH was sensitive enough to readily identify a dosage gain for clones contained on a small marker chromosome 17 present in 72% of cells. Interestingly, while a handful of clones (some containing LCRs) within the array performed less well than the majority when patient to control fluorescence ratios were analyzed, several clones that contain large LCRs did not have an adverse effect on the interpretation of the array-CGH data. Our data demonstrate that array-CGH is an accurate and sensitive method for detecting genomic dosage differences and identifying rearrangement breakpoints, even in LCR-rich regions of the genome.

**Development of a full-coverage X-chromosomal BAC array for high-resolution screening of genomic alterations in patients with X-linked mental retardation.** *J. Veltman<sup>1</sup>, H. Yntema<sup>1</sup>, H. Arts<sup>1</sup>, D. Lugtenberg<sup>1</sup>, C. on Choy<sup>2</sup>, K. Osoegawa<sup>2</sup>, E. Huys<sup>1</sup>, W. van der Vliet<sup>1</sup>, H. Straatman<sup>1</sup>, B. Hamel<sup>1</sup>, P. de Jong<sup>2</sup>, H. Brunner<sup>1</sup>, A. Geurts van Kessel<sup>1</sup>, H. van Bokhoven<sup>1</sup>, E. Schoenmakers<sup>1</sup>.* 1) Human Genetics, UMC Nijmegen, Nijmegen, Netherlands; 2) BACPAC resources, Oakland, CA.

Approximately half of the mentally retarded patients have a genetic origin and a significant proportion thereof is due to defects on the X chromosome. The European XLMR consortium (Leuven, Paris, Tours, Berlin, Nijmegen) has established a cohort of 200 well-characterized families with nonspecific X-linked mental retardation (MRX) which are being studied by various approaches. This unique cohort has been instrumental for the isolation of 6 of the 9 MRX genes that are known to date. Since most of the cytogenetically visible X-chromosomal deletions have already been studied, novel methods are needed to efficiently identify small submicroscopic deletions and duplications in MRX patients. Identification of such aberrations will immediately result in novel candidate genes for MRX. The technology that we have focussed on is array-based comparative genomic hybridisation (arrayCGH). We have constructed a full-coverage X-chromosomal BAC array consisting of approximately 1600 clones. The sensitivity and specificity of the technology were tested in a series of normal versus normal control experiments and a series of patients with known chromosome X copy number changes (including an Xq26 duplication, several Xq21 deletions, an Xp and an Xpter deletion, two PLP duplications and an XXXY). The results show that our array allows the detection of copy number changes >50 kb on the human X chromosome.

This study clearly demonstrates the power of the arrayCGH technology. Deletion and amplification mapping can now be performed at the submicroscopic level and will allow high throughput identification of novel X-chromosomal regions harboring genes involved in X-linked mental retardation.

**Submicroscopic chromosomal abnormalities and large-scale polymorphisms in mental retardation patients detected by genome-wide microarray analysis.** *L.E.L.M. Vissers<sup>1</sup>, B.B.A. de Vries<sup>1</sup>, K. Osoegawa<sup>2</sup>, I.M. Janssen<sup>1</sup>, T. Feuth<sup>1</sup>, C. on Choy<sup>2</sup>, H. Straatman<sup>1</sup>, W. van der Vliet<sup>1</sup>, E.H.L.P.G. Huys<sup>1</sup>, A. van Rijk<sup>1</sup>, D. Smeets<sup>1</sup>, C.M.A. van Ravenswaaij-Arts<sup>1</sup>, N. Knoers<sup>1</sup>, I. van der Burgt<sup>1</sup>, P.J. de Jong<sup>2</sup>, H.G. Brunner<sup>1</sup>, A. Geurts van Kessel<sup>1</sup>, E.F.P.M. Schoenmakers<sup>1</sup>, J.A. Veltman<sup>1</sup>.* 1) Human Genetics, UMC Nijmegen, Nijmegen, Netherlands; 2) BACPAC resources, Oakland, CA.

Microdeletions and microduplications, not visible by routine chromosome analysis, are a major cause of human malformation and mental retardation. In order to detect these microdeletions, novel high resolution whole genome technologies are needed. Array-based comparative genomic hybridization allows such a high-resolution screening by hybridizing differentially labeled test and reference DNAs to arrays consisting of thousands of genomic clones. In this study we tested the diagnostic capacity of this technology using approximately 3500 FISH-verified clones selected to cover the genome with an average of one clone per megabase. The sensitivity and specificity of the technology were tested in normal versus normal control experiments and through the screening of patients with known microdeletion syndromes. Subsequently, a series of 20 mentally retarded and dysmorphic patients with normal karyotypes were analyzed and 3 microdeletions and 2 microduplications were identified and validated. Two of these genomic changes were identified also in one of the parents, indicating that these are large-scale genomic polymorphisms. Deletions and duplications as small as 1 Mb could reliably be detected by our approach. The percentage of false positive results was reduced to a minimum by using a dye-swap replicate analysis, all but eliminating the need for laborious validation experiments and facilitating implementation in routine diagnostics. This high-resolution assay will facilitate the identification of novel genes involved in human mental retardation and/or malformation syndromes and provide insight in the flexibility and plasticity of the human genome.

**Analysis of the D21Z1 alphoid junction regions in the centromere of human chromosome 21.** *M. Bozovsky<sup>1</sup>, S. Shukair<sup>1</sup>, M. Puckelwartz<sup>1</sup>, M. Cummings<sup>2</sup>, J. Doering<sup>1</sup>*. 1) Dept. of Biology, Loyola University Chicago; 2) Dept. of Biological Sciences, University of Illinois at Chicago.

The centromeric and other heterochromatic regions that constitute 10-15% of the human genome have not been included in the completed genome sequence. Sequences required for a functional centromere have not been fully defined, but alphoid repetitive sequences clearly play a role. D21Z1, the major alphoid cluster of HC21 is a 1.0Mb long homogenous array with a higher-order repeat structure and regular CENP-B boxes. A 0.3Mb long heterogeneous satellite-I cluster is located 0.25Mb from the p-arm end of D21Z1. Characterizing the ends of the alphoid array may clarify evolutionary events in centromere formation. We have identified YAC clones that contain the D21Z1 p-arm junction. The physical structure of the alphoid DNA present in this region suggests a gradual loss of alphoid sequence homogeneity as one moves closer to the end of the array. In contrast, the physical structure of the q-arm junction region suggests an abrupt alphoid/non-alphoid transition. Sequence analysis of the q-arm junction has been done using BAC clone 21B49A22, the last clone in the q-arm sequence, which contains 31.2kb of alphoid DNA adjacent to non-repetitive DNA. This alphoid DNA is highly heterogeneous, showing as little as 74% sequence similarity between monomers, and is devoid of both regular CENP-B boxes and higher-order repeat structure. The unequal crossing over model of tandem array evolution predicts a similar structure at both array ends, but our data on D21Z1 suggests that the two junction regions have very different organizations from one another.

The monomeric alphoid array at the D21Z1 q-arm junction seems to have been established and evolved independently of the D21Z1 array and may therefore have HC21 specific sequences. We have subcloned a region of the q-arm monomeric alphoid array that is polymorphic and which BLAST analysis indicates may be chromosome specific. An HC21 specific centromeric marker would be useful in diagnosing aneuploidy and permit more accurate scoring of parent of origin and stage of meiosis of a nondisjunction event.

**An estimate of telomere length for an acrocentric short arm.** *H. Dimitropoulos<sup>1</sup>, S.M. McCutcheon<sup>1</sup>, Y. Ahn<sup>1</sup>, J. Doering<sup>2</sup>, M.R. Cummings<sup>1</sup>.* 1) Department of Biological Sciences, University of Illinois, Chicago, Chicago, IL; 2) Department of Biology, Loyola University, Chicago, IL.

Telomeres protect the ends of human chromosomes in normal cells and are composed of the tandem hexameric repeat (TTAGGG)<sub>n</sub>. Each chromosome end contains a variable number of these repeats that contribute to a characteristic telomere length ranging from 2-12 kb. Shortening of telomeres has been linked to cellular events such as aging and genetic instability. Factors that contribute to the size of a particular telomere include chromosome size, proximity of the centromere, the subtelomeric region and telomerase activity within the cell. Acrocentric short arm telomeres and subtelomeric regions have not been included in mapping and sequencing projects. Our laboratories are creating a physical map of the distal tip of the human chromosome 21 short arm, spanning the region from the rDNA to the short arm telomere. This region can be separated into the telomere-containing region and a subtelomeric domain that contains over a dozen different types of repetitive sequences. We used two separate approaches to estimate telomere length of the distal tip: terminal restriction fragment (TRF) analysis and Bal31 exonuclease digestion of DNA from somatic cell hybrids carrying the p arm of chromosome 21. Our findings from both assays show that the p arm distal tip contains only about 1 kb of the (TTAGGG)<sub>n</sub> sequence and provide the first estimate of telomere length for an acrocentric short arm. Our findings are in agreement with studies in other mammals that show that the closer a telomere is to the centromere, the shorter the telomere is likely to be. Additionally, recent evidence shows that it is the shortest telomere in a cell that may be critical in controlling cell fate. Our data suggests that telomere length on the p arm of chromosome 21 and/or other acrocentric chromosomes may have a significant functional role in phenomena such as cellular aging and genetic instability.

Program Nr: 1505 from 2003 ASHG Annual Meeting

**Identifying matrix attachment regions in human genome using microarray-based approach.** *Z. Jiang<sup>1</sup>, Z. Yuan<sup>1</sup>, L. Jin<sup>1,2</sup>*. 1) Dept. Environmental Health, University of Cincinnati, Cincinnati, OH; 2) School of Life Sciences, Fudan University.

Identification of functional and structural DNA elements is important in annotating the sequence of the human genome. One of these elements, the matrix attachment region (MAR) is a basic component of chromosome loop structure and was reported to play an important role in DNA replication, gene transcription, recombination and DNA repair. We developed a microarray-based method which allows a reliable and highthroughput detection of all MARs in the human genome. The validation of the method was conducted by successfully identifying all four MARs that have been reported in a 200-kb region containing TOP1 gene.

**Extended haplotype block structure and evidence for selection in a 900 kb region of the ATM Gene in human and chimpanzee.** *Y.R. Thorstenson<sup>1</sup>, P. Shen<sup>1</sup>, D.P. Wall<sup>2</sup>, T.L. Wayne<sup>1</sup>, V. Chou<sup>1</sup>, R.W. Davis<sup>1</sup>, P.J. Oefner<sup>1</sup>.* 1) Stanford Genome Technology Ctr, Palo Alto, CA; 2) Harvard Medical School, Boston, MA.

The ATM gene product is an important regulator of cell cycle control and DNA repair in mammalian cells. Mutations in the gene are known to cause the cancer-prone, recessive genetic disease ataxia-telangiectasia. Previous haplotype analysis at ATM indicated it was part of an extended haplotype block. To delineate the boundaries of the ATM haplotype block, SNP markers were identified across a 900 kb region encompassing the genes ATM, NPAT, ACAT1, CUL5 and SLAC2\_B in both human and chimpanzee. The markers were then genotyped in 34 African and 154 non-African chromosomes, and in 24 chimpanzee chromosomes. Pairwise  $|D'|$  patterns were constructed and plotted using unphased genotype data. A strong LD covering both the ATM and NPAT genes was found both in African and non-African samples, defining an unusually large haplotype block of around 300kb. Analysis of the same region in chimpanzee revealed an LD block of around 180kb, which covered only the ATM gene region. This unusually large LD block in ATM region (vs. an average of 24kb across the genome) indicated the presence of either a recombination cold-spot, or selection forces acting on this gene. To test the hypothesis of selection, Yang's likelihood ratio test was performed to estimate the rates of synonymous and nonsynonymous substitutions in the primate lineages using the orthologous ATM coding sequences for human, chimp, gorilla, orangutan, macaque, patas monkey and mouse. A significantly lower rate of change of non-synonymous versus synonymous sites observed in ATM is consistent with purifying selection across all primate lineages.

**A New Method for Amplification and Labeling of RNA from Small Clinical Samples for Use with the Affymetrix GeneChip Platform.** *G. Deng, A. Dafforn, M. Wang, P. Chen, R. Purohit, S. Wang, S. Pillarisetty, D. Iglehart, S. Koritala, S. Lato, M. Herrler, J. Heath, J. Stanchfield, N. Kurn.* NuGEN Technologies, Inc., San Carlos, CA 94070.

Microarray analysis is a powerful technology for profiling the large-scale changes in gene expression that occur as part of both normal and disease state biological processes. However, the application of the technology has been limited by the need for relatively large amounts of RNA. Most clinical RNA samples are derived from minute amounts of tissue, such as obtained by fine needle aspiration or laser capture microdissection, and require RNA amplification. Most available RNA amplification methods are tedious and time-consuming. There is a need for simple amplification methods suitable for automation and high throughput analysis. The RNA amplification and labeling system developed by NuGEN is based on a novel linear, rapid and sensitive amplification method, Ribo-SPIA. This system generates micrograms of cDNA from nanogram amounts of starting total RNA in less than four hours. The Ribo-SPIA based amplification and labeling system for the generating labeled amplified cDNA for microarray analysis with dual channel detection was used successfully on a variety of microarray platforms (home-brew spotted oligonucleotide and cDNA arrays, Agilent printed arrays). We have recently developed methods of fragmenting and chemically coupling biotin to Ribo-SPIA products, as required for Affymetrix GeneChip and Amersham CodeLink arrays. The Ribo-SPIA based system generates highly reproducible array results on both platforms. GeneChip analysis revealed higher sensitivity afforded by Ribo-SPIA amplified cDNA (prepared from 20ng input total RNA) as compared to cRNA prepared by Affymetrix standard protocol (20ug input total RNA). Spike detection experiments demonstrate that Ribo-SPIA has a wide linear amplification range, which is critical for maintaining high fidelity of message representation. We have successfully applied this amplification technology to examine gene expression in a variety of clinical samples including tissue biopsies.

**Whole genome amplification for genotyping, sequencing, and methylation analysis-Applications in genomics, pharmacogenomics, diagnostics, biosurveillance, and forensics.** *J. Langmore.* Rubicon Genomics, Inc., Ann Arbor, MI.

Many genetic studies and diagnostic tests are limited by the amount of DNA or RNA that is available for study. Common factors that lead to inadequate amounts of nucleic acid are small initial sample size, degradation at the source or in handling/storage, cost, depletion of existing samples by unanticipated numbers of tests or collaborators, and difficulties of recontacting subjects. Cell transformation and growth is not a viable solution to these problems. We have developed a simple method for whole genome amplification, called OmniPlex WGA, that can accurately and robustly amplify sub-nanogram amounts of total human DNA using common reagents. The process is a random, non-enzymatic fragmentation of genomic DNA followed by addition of adaptor sequences to both ends to form an in vitro molecular library that is amplified using PCR. Library preparation and amplification takes less than three hours, is automatable, and can be repeated multiple times to produce milligram amounts of DNA. OmniPlex WGA data will be shown from whole blood, blood spots, buccal swabs, serum, fixed or frozen tissue, hair follicles, degraded archived DNA, single cells, and single sorted chromosomes. OmniPlex has been used in academic, government, and commercial projects for SNP and STR genotyping, for mutation discovery by sequencing and heteroduplex analysis, for chromosome painting and CGH, and for methylation and expression analysis. Genotype concordance between the gDNA and the WGA DNA is >99.7% on high throughput single base extension, ligation, and exonuclease assays. By enabling large-scale genotyping or resequencing studies to be done with blood spots, hair, or buccal swabs, WGA allows genetic resources for large-scale population studies to be collected, archived, and shared more rapidly and economically than by other methods. The very low background, insensitivity to DNA breakage, and high sequence accuracy of the amplification process make WGA an attractive method for more accurate and sensitive genetic and epigenetic diagnostics, and human and pathogen identification.

**Comparative genomic analysis of the HNF-4 transcription factor gene.** *A.M. Bagwell<sup>1</sup>, A. Bailly<sup>3</sup>, J.C. Mychaleckyj<sup>1</sup>, B.I. Freedman<sup>2</sup>, D.W. Bowden<sup>1</sup>.* 1) The Center for Human Genomics; 2) Department of Internal Medicine, Wake Forest Univ Sch of Med, Winston-Salem, NC; 3) Pasteur Institute, Paris, France.

A positional candidate gene in the type 2 diabetes mellitus (T2DM) linked region on chromosome 20q12-q13.1 is hepatocyte nuclear factor-4 alpha (HNF-4), the gene for the Maturity-Onset Diabetes of the Young type 1 (MODY1) sub-type of T2DM. Mutations in the coding region of HNF-4 are rare in T2DM individuals. We hypothesized that altered regulation of HNF-4 gene expression may contribute to susceptibility to T2DM. Comparative genomic analysis using PipMaker was performed with 13kb of 5' promoter sequences of human, mouse, and rat HNF-4. Three regions, located at -10.344kb (295bp in length), -6.476kb (421bp in length), and -5.472kb (263bp in length), were identified as having significant sequence identity between the 3 species with expect values of  $1e-37$ ,  $1e-84$ , and  $1e-79$ , respectively. Functional analysis of these regions using transient transfection assays revealed that the sequences have weak ability to activate gene expression in pancreatic cells or dedifferentiated hepatoma cells, with expression being 1.0-2.5-fold over control. In differentiated hepatoma cells, the 295bp region and the 263bp region exhibit weak, though statistically significant, activity over control ( $P=0.001$ ). The construct containing the 421bp sequence confers strong activity in the differentiated hepatoma cells, with a  $13.71 \pm 1.95$ -fold increase in reporter gene activity over control vector ( $P=0.006$ ), suggesting that the 421bp sequence is an enhancer of HNF-4 gene expression that acts in a tissue-specific manner. The DNA of 259 Caucasian T2DM subjects was screened by denaturing high performance liquid chromatography to identify sequence variants in the conserved regions. Only 2 SNPs were identified, both of which increased reporter gene activity 1.42-1.70-fold over wild type in the insulinoma cell lines ( $P=0.05$ ). These results suggest that functional regulatory elements of HNF-4, and likely other genes, can be identified by comparative genomic analysis. Furthermore, the limited sequence variation within these regions suggests that these elements are likely of functional importance in humans.

**An ENU mutagenesis program using mice for identifying genes controlling complex traits.** *P. Charmley<sup>1</sup>, M. Appleby<sup>1</sup>, K. Staehling-Hampton<sup>1</sup>, N. Wiegand<sup>1</sup>, J. Gilchrist<sup>2</sup>, F. Ramsdell<sup>1</sup>, J. Bouck<sup>1</sup>, T. Britschgi<sup>1</sup>, A. Snell<sup>1</sup>, T. Howard<sup>1</sup>, M. McEuen<sup>1</sup>, B. Paeper<sup>1</sup>, S. Proll<sup>1</sup>, P. Tittel<sup>1</sup>, G. Carlson<sup>2</sup>, R. Schatzman<sup>1</sup>, M. Brunkow<sup>1</sup>.* 1) Department of Genomics, Celltech R&D, Inc., Bothell, WA; 2) McLaughlin Research Institute, Great Falls, MT 59405.

ENU mutagenesis in mice is being used to dissect aspects of complex traits in clinically relevant areas such as lymphocyte biology, inflammation and autoimmunity. The particular approach we are using involves a three-generation recessive screen, focusing on phenotypes which mimic desired clinical responses (e.g., suppressed inflammatory response), thus improving our chances of directly identifying relevant therapeutic targets. We have implemented a number of in vitro screens including lymphocyte activation, as well as T-dependent and T-independent inflammatory responses. These are carried out on peripheral blood lymphocytes, and have the advantage of being relatively high throughput and requiring only small volumes of blood, thus affording us the opportunity to perform a number of manipulations on a single sample. The in vitro screens have been coupled with a complementary set of more complex in vivo screens based on classic pharmacologic models of inflammation and immune response (e.g. contact sensitivity). In instances where tissue samples can be collected (e.g. ear punches), we have also applied gene expression technologies to study inherited variation on a per-gene basis. In the past 3 years, over 100 phenodeviants have been identified and entered into our mapping process. We have developed an integrated laboratory / informatic pipeline which enables rapid identification of a candidate interval and the genes contained within, as well as efficient tracking of gene testing results, capturing both DNA sequence and gene expression data. The development and utilization of informatics tools has proven critical in the effective management of the program. Another important aspect of the program is the ongoing process of new screen development to ensure as broad an interrogation of the immune system as possible. Specific lessons learned from the mutations identified so far will be discussed in more detail.

**Autosomal localization of mouse X-linked genes in the rat.** *B.-Y. Liao*<sup>1</sup>, *S. Tyan*<sup>1</sup>, *M.-J. Hwang*<sup>1</sup>, *P. Yen*<sup>1,2</sup>. 1) Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Department of Pediatrics, Harbor-UCLA Medical Center, Torrance, California, U.S.A.

Ohno's law of the conservation of the X chromosome in placental mammals predicts that genes on the X chromosome of one mammalian species will be X-linked in all mammals due to X-inactivation. Translocations between autosomes and the X chromosome during evolution would result in dosage imbalance and be detrimental to the species. So far this law has been observed on most X-linked genes, with the exceptions being genes located within or near the pseudoautosomal regions. In a recent blast of the rat draft genome sequence at NCBI, we unexpectedly found autosomal assignments of several mouse genes from different regions of the X chromosome. Orthologues of these mouse X-linked genes were located on the supercontigs of several rat chromosomes, including chromosomes 1, 4, 5, 6, 7, and 8. To investigate whether and when these X-autosomal translocations have occurred, we performed Southern hybridization/dosage analysis on several rodent species including lab mouse (*Mus musculus*), wood mouse (*Apodemus semotus*), rat (*Rattus norvegicus*), and Syrian hamster (*Mesocricetus auratus*). Comparison of the signal intensities between male and female animals provided unambiguous assignments of the genes to the X or the autosomes when known X-linked and autosomal genes were used as controls. We failed to confirm the autosomal localizations, as indicated in the rat draft genome sequence, for the few genes tested. Our results have uncovered significant errors in the assembly of the rat genome that used a hybrid of the whole genome shotgun approach and the hierarchical BAC clone approach. These errors are likely caused by the presence of highly homologous sequences on different chromosomes.

**Interactive Locus Specific DataBases for muscular dystrophies.** *C. Beroud*<sup>1</sup>, *J.A. Urtizbera*<sup>2</sup>, *J.C. Kaplan*<sup>3</sup>, *M. Claustres*<sup>1</sup>. 1) Laboratoire de Genetique, Hosp Arnaud de Villeneuve, Montpellier, France; 2) Hosp Raymond Poincare, 92380 Garches, France; 3) Department GDPM, Institut Cochin, 75014 Paris, France.

In addition to **general** mutation databases, **locus specific** mutation databases are needed to confront molecular and clinical data. The **UMD** (*Universal Mutation Database*) generic software (*Beroud et al, Human Mutation 2000*) was created to develop Internet accessible Locus Specific DataBases (LSDB). We are currently applying this concept to 9 muscular dystrophy genes: *DMD*, *LMNA*, *EMD*, *LAMA2*, *SGCA*, *SGCG*, *CAPN3*, *DYSF* and *FKRP*. The **UMD-DMD** database (dystrophinopathies) comprises the entire fully annotated genomic sequence encompassing the *DMD* gene. The software features *DMD*-specific packages such as automatic computation of the impact of the mutation upon: (i) the reading frame, (ii) the consensus value of splice sites, (iii) the theoretical size of transcripts and translational products, (iv) the loss of epitopes, polymorphic markers and critical protein domains. In addition it computes all possible exon-skipping patterns capable of restoring the frame and compatible with a functional dystrophin. It also provides graphical display of the various deletions and duplications. The **UMD-DMD** accommodates mutational events occurring deep in the huge introns (cryptic exon activating point mutations, deletion/duplication intronic breakpoints etc). The current version of the database contains 800 unrelated patients with fully documented mutations in the *DMD* gene. **UMD-LSDBs** are not inert repositories but interactive tools using the power of computer analysis to answer complex queries such as phenotypic heterogeneity and genotype/phenotype correlations. They will help to pinpoint discrepant cases in which the clinical tolerance differs from that expected. They should help elaborate allele-specific gene-based therapeutic strategies. One drawback of **LSDBs** is that one has to query various databases before knowing which gene is associated with a specific phenotype. To solve this problem, we are developing the **UMD-central system** which is able to query all **UMD-LSDBs** and give an overview of their data. For this, we have developed a standard clinical submission form for all **UMD** muscle dystrophy **LSDBs**.

**A Novel Method for Building Neuropsychiatric Candidate Gene Sets and SNP Maps for Association Studies.**

*K.J.L. Irizarry<sup>1</sup>, A. Day<sup>2</sup>, K. Mitsouras<sup>2</sup>, C.J. Lee<sup>3</sup>, J. Licinio<sup>1</sup>*. 1) NeuroPsychiatric Insititute, UCLA, Los Angeles, CA; 2) Department of Human Genetics, UCLA, Los Angeles, CA; 3) Department of Chemistry & Biochemistry, Laboratory of Bioinformatics & Structural Proteomics, UCLA, Los Angeles, CA.

We describe a novel method for producing candidate gene sets which rescues many false negatives and simultaneously reduces the addition of false positives. Such a gene set can be of considerable value for use in disease gene association studies. We applied this approach to the human genome to produce a neuropsychiatric candidate gene set with cSNPs enriched for protein functional impact. We successfully identified 906 neuronal signaling genes which together contain 1917 SNPS. We have assessed the impact of non conservative cSNPs on protein function by analyzing such SNPs when they occur with a functionally important domain. In addition we performed two independent verifications to assess, first the association of genes exhibiting non-conservative cSNPs with known disease genes. We searched for published disease annotation among 18 genes and identified 15 that have been implicated in one or more diseases. Secondly, we mapped our 906 candidate gene set to 61 schizophrenia associated loci from OMIM and identified 208 genes and with 497 SNPs. This candidate gene set, together with the identified SNPs and comprehensive schizophrenia map represent a valuable resource for genetic association studies of diseases affecting the human nervous system.

**INTEGRATING GENETICS WITH GENOMICS AS A TOOL TO UNRAVEL MOLECULAR PATHWAYS IN CELIAC DISEASE.** *C. Wijmenga, M. van Belzen, B. Diosdado, A. Zhernakova, A. Monsuur, A. Bardoel, L. Franke, M. Wapenaar.* Dept Biomedical Genetics, Univ Medical Ctr, Utrecht, Netherlands.

Celiac disease (CD) is a complex disease of the small intestine induced by gluten. CD is common with a prevalence of ~1:200 in Western populations. The causative molecular pathways underlying celiac disease pathogenesis are poorly understood. To unravel novel aspects of disease pathogenesis, we use both genetic linkage studies and microarray analysis of duodenal biopsies. A major known susceptibility locus for CD is the HLA DQ locus on chromosome 6p. Recently we identified two additional risk loci, on chromosomes 6q and 19p. The 19p region covers some 3Mb and contains 92 known genes and ETSs. The region on 6q is ~10 Mb and contains 101 transcript. In addition, cDNA microarrays were used to compare gene expression profiles of duodenal biopsies from celiac disease patients with villous atrophy and control individuals with normal biopsies. We identified 109 genes that differed significantly ( $p < 0.001$ ) in expression levels between patients and controls. A large number of these genes have functions in proliferation and differentiation pathways and might be important for proper development of crypt-villous units. Alterations in this pathway may lead to the characteristic hyperplasia and villous atrophy seen in celiac disease. In order to combine genetic linkage data and microarray gene expression results, we developed a Java based computer program. This program includes a viewer, capable of showing karyobands, genes, markers, linkage graphs, gene expression levels, SNPs and additional annotation data simultaneously. Additionally data management and filtering functionality was implemented and the databases Ensembl, LocusLink, Gene Ontology and Unigene were integrated. Interestingly, the linkage peaks on 6q and 19p contain 11 and five differentially expressed genes, respectively. These genes are currently being followed up with a high density of SNPs.

**Accentuate the negative: Uniqueness in proteome comparisons among model organisms.** *E. Bier, M. Fischer, N. Hong, L. Reiter.* Department of Biology, University of California, San Diego, La Jolla, CA.

With the completion of the genome sequences of model genetic organisms we enter a new era of bioinformatics. The majority of amino acid sequence comparisons of model organisms to the human proteome have been aimed at identifying similar proteins or related protein domains. What has largely been under-studied, however, is the nature of proteins that are either unique to particular proteomes or are shared by some but not all organisms. These proteins may be essential in defining organismal identity. To identify the proteins unique to a given proteome BLAST analysis was performed between proteomes using the most current sets of proteins from *H. sapiens*, *M. musculus*, *D. melanogaster*, *C. elegans*, *S. cerevisiae*, *E. coli* and *A. thaliana*. Comparisons were done at the low stringency e-value of 1 in order to identify proteins that unquestionably did not match in a given comparison. This BLAST data was compiled in a flexible MySQL database for cross species associations: the [Negative Proteome Database](http://superfly.ucsd.edu/negprot). We used this database to identify sets of proteins which are unique to a given model organism, and may represent evolutionary novelty, such as genes regulating flowering in *A. thaliana*, genes conferring sensitivity to BT toxins in *C. elegans*, or proteins involved in pheromone response in *D. melanogaster*. We find that genes encoding structural components of the organisms outer covering as well as innate defense genes are highly represented in all annotated organism-specific gene sets. With regards to studying human disease gene homologs, the database now allows us to select the most appropriate model system for a given gene. This database allows the user to find positive matches to proteins not found in a given comparison as well. For example, one can identify all of the proteins in insects that are not in plants or in humans. These proteins may prove excellent targets for pesticides or insecticides since they are not in either of the organisms one wishes to protect (plants and humans).

**Comparative analysis of ribosomal protein genes and evolution of introns.** *N. Kenmochi, M. Yoshihama, T. Uechi, A. Nakao.* Research Center for Frontier Bioscience, Miyazaki Medical College, Miyazaki, Japan.

The ribosome, as a catalyst for protein synthesis, is universal and essential for all organisms. Because of the fundamental role played by ribosomes, their structure and function have been significantly conserved during evolution. In higher eukaryotes, the ribosome is composed of 4 RNA molecules (rRNAs) and 79 distinct proteins (RPs). We have compared the intron/exon structures of these genes from humans, *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Saccharomyces cerevisiae*. Because the size and sequence of the coding regions are very similar between the orthologs among these species (63% homology on average), it was easy to compare the intron positions in these genes.

In humans, we found 249 introns within the coding regions. Among them, the insertion sites of 137 (55%) were unique to the human genes; 77 (31%) were the same in humans and flies; and 60 (24%) were the same in humans and worms. Of these, 26 introns (10%) were common to all three species. By contrast, only 7 introns (3%) shared the same insertion sites in humans and yeast, and the position of only one, the second intron of *RPL14*, was conserved among all four species. About 80% of fruitfly introns were present in human RP genes, but only 30% of these introns appeared in worms.

Finally, we have constructed Ribosomal Protein Gene Database (RPG). It contains information about RP genes from above four species and *Methanococcus jannaschii* and *Escherichia coli*. Users can search the database by the gene name and organism. Each record includes various sequences (genomic, cDNA, and amino acid sequences), intron/exon structures, genomic locations, and information about orthologs. Also users can view gene structures from these organisms comparatively, and have multiple sequence alignments of amino acid sequences. RPG is available at <http://ribosome.miyazaki-med.ac.jp>.

Program Nr: 1517 from 2003 ASHG Annual Meeting

**ALFRED - the ALlele FREquency Database.** *H. Rajeevan<sup>1</sup>, M.V. Osier<sup>2</sup>, K. Cheung<sup>2</sup>, H. Deng<sup>1</sup>, L. Druskin<sup>2</sup>, J.R. Kidd<sup>1</sup>, S. Stein<sup>1</sup>, A.J. Pakstis<sup>1</sup>, N.P. Tosches<sup>2</sup>, P.L. Miller<sup>2</sup>, K.K. Kidd<sup>1</sup>.* 1) Department of Genetics; 2) Center for Medical Informatics, Department of Anesthesiology, Yale University School of Medicine, New Haven, CT 06520-8005 USA.

ALFRED (<http://alfred.med.yale.edu>) is an actively curated database designed to make allele frequency data on anthropologically defined human population samples readily available to the scientific community and to link these polymorphism data to the various molecular genetics-human genome databases. Users can search for frequency data through several search options including locus symbol, site name and a specific author's publications. Data may be viewed both as numeric tables and graphically. ALFRED is rapidly expanding and has doubled in size in the past year to 12,000 frequency tables (one population typed for one site or haplotype) involving 737 polymorphisms and 358 populations. All of the data in ALFRED are considered to be in the public domain and available for use in research and teaching. Researchers can download data useful for analysis in a variety of formats including a single compressed "data dump" file or separate files of relevant tables, in the declared XML format. The Data Type Definition (DTD) for validating the XML format is also available for download. The existing data are primarily those extracted from publications by curatorial staff or made available from a few laboratories. New data and new links to other databases are routinely being added to ALFRED. Presently addition of new data is focussed on popular and user-suggested polymorphic sites to help researchers to conduct comprehensive analyses. We now have systems in place to accept data directly from researchers. While XML data or a spreadsheet with the necessary descriptive information in specific columns are preferable, we can also accept data in most any electronic form if the descriptive information is submitted in parallel.

(Ongoing support of ALFRED is provided by NSF grant BCS-0096588).

**dbQSNP: a pipeline for SSCP based SNP-finding/quantification and publicizing allele frequency data of populations.** *T. Tahira*<sup>1</sup>, *S. Baba*<sup>1</sup>, *K. Higasa*<sup>1</sup>, *Y. Kukita*<sup>1,2</sup>, *Y. Suzuki*<sup>2</sup>, *S. Sugano*<sup>2</sup>, *K. Hayashi*<sup>1</sup>. 1) Res Ctr Genet Info, Med Inst Bioreg, Kyushu Univ, Fukuoka, Japan; 2) Human Genome Center, Inst Med Science, Univ. Tokyo, Tokyo, Japan.

We have developed a capillary-array-based single-strand conformation polymorphism (PLACE-SSCP) analysis, and shown that it is suitable for estimation of precise allele frequencies of SNPs using pooled DNA. In this method, allele frequencies of SNPs in the pool are determined from the peak heights of alleles separated by SSCP analysis. Here we present dbQSNP, a laboratory information management system that facilitates large scale SNP finding/allele frequency estimation by concerted analysis of SSCP and sequencing. dbQSNP is a relational database system, that uses PostgreSQL on the Unix server, and is managed through a WEB browser. Various newly developed as well as available algorithms are integrated. Data for each STS are summarized and publicized as a searchable record in a hypertext format, through which trace of SSCP and sequencing can be monitored. Using this system, we characterized SNPs in 1.2 kb genomic regions containing putative transcription start sites of various genes. SNPs are searched by examining 8 Japanese individuals, and allele frequencies of these SNPs are determined by quantifying peaks of pools (Japanese and Caucasian) after compensating for unequal peak heights in heterozygotes. We examined 4131 STSs (average size ca. 330 bp), found 1593 SNPs including 136 insertion/deletions, and half of the SNPs were new (not found in Build 115 dbSNP). Allele frequencies were successfully estimated for 86% of the STSs that contain single SNPs. Among 866 SNPs that showed more than 10% minor allele frequency in Japanese, 453 showed significantly different ( $p < 0.01$ ) allele frequency between Japanese and Caucasian. In addition, several of them showed high  $F_{st}$  values. Genetic differentiation in these particular sites after population subdivision is suspected.

**Human Protein Reference Database: Building a biological platform for systems biology.** *S. Peri<sup>1</sup>, J.D. Navarro<sup>1</sup>, R. Amanchy<sup>1</sup>, T.Z. Kristiansen<sup>1,5</sup>, J.C. Jonnalagadda<sup>1,2</sup>, S. Vineeth<sup>2</sup>, V. Niranjana<sup>2</sup>, B. Muthusamy<sup>2</sup>, T.K.B. Gandhi<sup>2</sup>, M. Gronborg<sup>1, 5</sup>, N. Ibarrola<sup>1</sup>, C.V. Dang<sup>3</sup>, J.G.N. Garcia<sup>3</sup>, J. Pevsner<sup>4</sup>, O.N. Jensen<sup>5</sup>, K.S. Deshpande<sup>2</sup>, A.M. Chinnaiyan<sup>6</sup>, A. Hamosh<sup>1</sup>, A. Chakravarti<sup>1</sup>, A. Pandey<sup>1</sup>.* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Institute of Bioinformatics, Innovator Tech Park Ltd., Bangalore, India; 3) Department of Medicine, Johns Hopkins University, Baltimore, MD; 4) Kennedy Krieger Research Institute, Johns Hopkins University, Baltimore, MD; 5) University of Southern Denmark, Odense, Denmark; 6) Department of Pathology, University of Michigan, Ann Arbor, MI.

Human Protein Reference Database (HPRD) is an object database that integrates a wealth of information relevant to the function of human proteins in health and disease. Data pertaining to protein-protein interactions, post-translational modifications, enzyme-substrate relationships, disease associations, tissue expression and subcellular localization were extracted from the literature for a non-redundant set of 2,750 human proteins. This includes all genes that encode proteins implicated in human diseases as annotated in the Online Mendelian Inheritance in Man (OMIM) database. Almost all the information was obtained manually by biologists who read and interpreted over 300,000 published articles during the annotation process. The database, which has an intuitive query interface allowing easy access to all the features of proteins, was built using open source technologies and is freely available at <http://www.hprd.org>. It documents over 10,000 protein-protein interactions and 1,900 post-translational modifications with over 25,000 links to PubMed. This unified bioinformatics platform will be useful in cataloging and mining the large number of proteomic interactions and alterations that will be discovered in the post-genomic era.

**A survey of housekeeping gene expression during germ cell development.** *A.L.Y. Pang<sup>1</sup>, W. Johnson<sup>1</sup>, N. Ravindranath<sup>2</sup>, M. Dym<sup>2</sup>, O.M. Rennert<sup>1</sup>, W.Y. Chan<sup>1,2,3</sup>.* 1) Laboratory of Clinical Genomics, NICHD, National Institutes of Health, Bethesda, MD; 2) Department of Cell Biology, Georgetown University, Washington, DC; 3) Department of Pediatrics, Georgetown University, Washington, DC.

With the advance of genomics technologies, studies attempting to identify the housekeeping (or maintenance) genes expressed in a variety of fetal and adult tissues have been performed recently. However, similar studies in specific cell lineages remain limited. Spermatogenesis is a tightly regulated developmental process of male germ cells; the intrinsic changes that occur during the transition from mitosis to meiosis as well as the rapid change in cell morphology underscore the existence of a sophisticated network of gene expression. In this study quantitative polymerase chain reaction (QPCR) was used to examine the expression of eighteen commonly known housekeeping genes at three distinct stages of mouse germ cell development (viz. type A spermatogonia, pachytene spermatocytes and round spermatids) along the spermatogenic pathway. Two of the genes (ubiquitin C and histone deacetylase 2) showed insignificant fluctuations in transcript levels among the three types of cells, while the other genes showed differences in the magnitude of their expression levels in the three stages. Besides these two genes, the 18S rRNA content was also found to display minimal changes between any types of germ cells examined. We concluded that the previously described two genes or 18S rRNA can be used as normalization markers for further gene expression studies during spermatogenesis in mouse germ cells. The constant expression of such genes along the spermatogenic pathway implies their indispensable role/activity in the germ cells.

**Mutational analysis of multiple developmentally important genes in the human condition Septo-Optic Dysplasia.**

*K.S Woods*<sup>1</sup>, *C. McCaffery*<sup>2</sup>, *J.P.G. Turton*<sup>1</sup>, *M.T. Dattani*<sup>1</sup>. 1) Biochem/Endoc/Metabolism Unit, Inst Child Health, London, United Kingdom; 2) London Genetics Knowledge Park, Institute of Child Health, UK.

Development of the anterior pituitary gland is a multigenic process regulated by complex signalling pathways which determine expression and interaction of a number of transcription factors. Fate map analysis reveals that the anterior neural ridge will give rise to the anterior pituitary whereas adjacent regions of the neural plate give rise to the posterior pituitary, optic vesicles and ventral forebrain. Previously, we described an association between septo-optic dysplasia (SOD), a human disease characterised by midline forebrain abnormalities, pituitary dysplasia and optic nerve hypoplasia and mutations in the homeobox gene *HESX1*<sup>1</sup>. However these mutations only account for a small number of cases of SOD (8/570). We have screened a large cohort (n=240) of SOD patients with variable phenotypes for candidate genes implicated in forebrain and pituitary development. The genes selected for this study (*EMX2*, *PAX6*, *NTNI*, *SIX3* and *SIX6*) were chosen on the basis of developmental expression pattern, information from mouse studies and previous association with genetic disorders affecting similar tissue types. Previously, we described a number of sequence variants, both exonic and intronic. None of the variations altered an amino acid residue and yet we were reluctant to dismiss any as non-pathogenic polymorphisms. To classify the importance of the sequence variations we identified, we utilised pyrosequencing technology. Using a panel of 94 Caucasian controls we were able to ascertain the frequency of each of these changes (*PAX6* IVS12 +42 t>g; +/+ 18%, +/- 54%, -/- 28%. *NTNI* IVS3 41c>t; +/+ 44%, +/- 44%, -/- 12% and *NTNI* IVS4 5 c>a; +/+ 42%, +/- 42%, -/- 16%). These results suggest that these sequence variants are indeed non-pathogenic polymorphisms. However non-pathogenic polymorphisms have been associated with functional consequence, e.g. an N125S polymorphism within *HESX1*<sup>2</sup>. Documentation of these polymorphisms will establish whether any, all or a combination of these variations may contribute to the SOD phenotype.

**Understanding the evolution of trypsinogen activation peptides through integration of functional characterization of disease-associated mutations with comparative genomic analysis.** *J.M. Chen<sup>1</sup>, Z. Kukor<sup>2</sup>, C. Le Maréchal<sup>1</sup>, M. Tóth<sup>3</sup>, L. Tsakiris<sup>4</sup>, O. Raguénès<sup>5</sup>, C. Férec<sup>1,5</sup>, M. Sahin-Tóth<sup>2</sup>.* 1) Institut National de la Santé et de la Recherche Médicale 01 15, Génétique Moléculaire et Génétique Epidémiologique, Université de Bretagne Occidentale, Etablissement Français du Sang-Bretagne, Brest 29220, France; 2) Department of Molecular and Cell Biology, Boston University Goldman School of Dental Medicine, Boston, Massachusetts 02118, USA; 3) Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest, Hungary; 4) Centre Hospitalier Universitaire de Melun, Service de Gastroenterologie, Melun, France; 5) Centre Hospitalier Universitaire de Morvan, Brest, France.

The activation peptide of mammalian trypsinogens contains a highly conserved tetra-aspartate sequence (D19-D20-D21-D22) preceding the K23-I24 scissile peptide bond, which is hydrolyzed as the first step in the activation process. Here we examined the evolution and function of trypsinogen activation peptides through integrating functional characterization of disease-associated mutations with comparative genomic analysis. Activation properties of three pancreatitis-associated activation peptide mutants (D19A, D22G and K23R) were simultaneously analyzed, for the first time, in the context of recombinant human cationic trypsinogen. A dramatic increase in autoactivation of cationic trypsinogen was observed in all three mutants, with D22G and K23R exhibiting the most marked increases. The physiological activator enteropeptidase activated the D19A mutant normally; the D22G mutant was activated very poorly, while activation of the K23R mutant was stimulated. The biochemical and structural data, taken together with a comprehensive sequence comparison, indicates that the tetra-aspartate sequence in mammalian trypsinogen activation peptides has evolved not only for optimal enteropeptidase recognition in the duodenum, but also for efficient inhibition of trypsinogen autoactivation within the pancreas. Moreover, the use of Lys instead of Arg at the P1 position of activation peptides also has an advantageous effect against trypsinogen autoactivation.

**Concerted Evolution in Mammals of Talanin and Uricase Genes.** *F. Gianfrancesco*<sup>1,2</sup>, *T. Esposito*<sup>1,2</sup>, *G. Maninchedda*<sup>2</sup>, *G. Casu*<sup>1</sup>, *M. Rocchi*<sup>3</sup>, *M. Pirastu*<sup>1,2</sup>. 1) Institute of Population Genetics, CNR, Alghero, Italy; 2) Shardna Life Science, Cagliari, Italy; 3) Institute of Genetics, University of Bari, Italy.

Recently, we identified a susceptibility locus for human Uric Acid Nephrolithiasis (UAN) on 10q21-q22 and demonstrated that a novel gene (ZNF365) included in this region produces through alternative splicing, 4 different transcripts: A, B, C and D coding for different protein isoforms. Mutation analysis showed that one of them (Talanin) is associated with UAN. The comparative genomic approach can be instrumental for understanding the role of Talanin in uric acid metabolism. Searching for mouse homologs of ZNF365 transcripts, we identified a highly conserved mouse ortholog of ZNF365A transcript, expressed specifically in brain. We did not find a mouse homolog for ZNF365D transcript highly expressed in kidney, encoding the Talanin protein, even if we were able to identify the corresponding genomic region in mouse and rat not yet organized in canonical intron-exon structure suggesting that ZNF365D was originated after the branching of hominoid from rodent lineage. Mouse and most mammals have a functional uricase that degrades the uric acid to allantoin while in hominoid the uricase activity was lost during the Miocene epoch. Analyzing the presence of Talanin in Primates, we found in Old World and New World monkeys a canonical intron-exon structure but with so many mutations that protein production was in fact prevented. In nonhuman hominoids we detected expression at extremely low levels. In human we observed higher expression and evidence that ZNF365D transcript produces a functional protein. It seems therefore that ZNF365D transcript emerged during primate evolution from a noncoding genomic sequence that evolved in a standard gene structure and assumed its role in parallel with the disappearance of uricase, probably against a disadvantageous excessive hyperuricaemia. The interplay of these two processes, indicates a possible physiological role of Talanin in uric acid excretion as also suggested by its association to UAN. Our findings demonstrate that the analysis of gene evolutionary processes may help understand the functions of genes in physiological and metabolic pathways.

**Genome-wide comparative sequence analysis between human, mouse, and chimpanzee identifies human genes under positive selection.** *M. Cargill*<sup>1</sup>, *A. Clark*<sup>2</sup>, *S. Glanowski*<sup>3</sup>, *R. Nielsen*<sup>2</sup>, *D. Tanenbaum*<sup>4</sup>, *P. Thomas*<sup>5</sup>, *J. Duff*<sup>4</sup>, *H. Gire*<sup>3</sup>, *S. Ferreira*<sup>3</sup>, *D. Civello*<sup>1</sup>, *C. Evans*<sup>3</sup>, *C. Fosler*<sup>3</sup>, *C. Forbes*<sup>3</sup>, *S. Hannenhalli*<sup>3</sup>, *A. Kejariwal*<sup>5</sup>, *S. Levy*<sup>4</sup>, *M. Rydland*<sup>3</sup>, *S. Schrodi*<sup>1</sup>, *J. Sninsky*<sup>1</sup>, *M. Adams*<sup>4</sup>. 1) Celera Diagnostics, Alameda, CA; 2) Cornell University, Ithaca, NY; 3) Applied Biosystems, Rockville, MD; 4) Celera Genomics, Rockville, MD; 5) Celera Genomics, Foster City, CA.

Genome-wide sequence comparison of human and chimpanzee genes in the context of mouse can serve as a powerful filter to identify genes that show strongly divergent patterns of sequence change. Furthermore, the completion of the human genome sequence allows an opportunity for focused exploration of particular regions of the genome for human variation. Studies of human variation and sequence divergence from other species will provide markers for use in genetic studies and potentially provide insight into human phenotypes. Recently, we completed a project in which we designed over 200,000 PCR amplicons to 23,000 human genes and sequenced the amplified products in 39 human individuals and 1 chimpanzee. This large data set encompassing 18M sequence reads yielded 265,000 human polymorphisms and ~33Mb of human (~25Mb chimpanzee) genomic sequence. We performed an evolutionary sequence analysis on three data sets derived from this project: 7,645 human-mouse-chimp orthologous coding sequence trios; 20,000 human-chimp coding sequence alignments; and 5Mb human-chimp-mouse non-coding sequence alignments. Approximately 8% of genes in the human genome show evidence of adaptive evolution and this set of genes is enriched in genes causing Mendelian diseases. We will present specific human genes and classes of genes that show evidence of positive selection on the human lineage. Furthermore, the incorporation of human polymorphisms discovered from our re-sequencing effort will place into context the human-specific divergence present in the alignments. Finally, a genome-wide comparison of sequence divergence and polymorphism in putative transcription factor binding sites will be described.

**A mammalian chromosomal rearrangement involving a cis-regulatory element of the *Gdf6* gene: implications for duplication-mediated gene evolution and for gene regulatory boundaries.** *D.P. Mortlock*<sup>1</sup>, *R.L. Chandler*<sup>1</sup>, *K.J. McDermott*<sup>1</sup>, *M.E. Portnoy*<sup>2</sup>, *NIH Intramural Sequencing Center*<sup>2</sup>, *E.D. Green*<sup>2</sup>. 1) Vanderbilt University School of Medicine, Nashville, TN; 2) National Human Genome Research Institute, Bethesda, MD.

Duplicated stretches of mammalian genomic DNA, or duplicons, may catalyze evolution at both the gene and chromosomal levels. Here we provide evidence that a duplicon near the mammalian *Gdf6* gene led to a rodent-specific chromosomal rearrangement involving the *Uqcrb* gene and a *Gdf6* regulatory element. *Gdf6* is a BMP family gene required for development of multiple skeletal joints. Previously, we described BAC transgenes that revealed a regulatory region 5' to *Gdf6* which is required for *Gdf6* transcription in limb joints. To study *Gdf6* evolution, we sequenced *Gdf6* BACs from baboon, chimp, cat, dog, cow, opossum and platypus, then performed comparisons with mouse, rat and human *Gdf6* genomic sequences. MultiPipMaker comparisons showed *Gdf6* locus conservation across all non-rodent mammals, and *Uqcrb* as the closest 5' flanking gene. However in rodents a synteny break relative to other mammals was found 70 kb 5' to *Gdf6*. Analysis of genome browser data revealed that in mice, *Gdf6* and *Uqcrb* are unlinked and on chromosomes 4 and 13, respectively. However, adjacent to both genes are copies of a 10 kb duplicon. Each duplicon maps to the end of a human synteny block. Furthermore, the duplicon mobilized part of the *Uqcrb* gene and a *Gdf6* cis-regulatory sequence. Therefore, duplicons can spread not only genes to new genomic regions but also regulatory elements, which potentially could confer novel regulatory effects on other genes. We propose that the duplicons catalyzed a rodent-specific chromosomal rearrangement. Although previous transgenic evidence suggests more *Gdf6* enhancers lie in distant regions flanking the gene, the duplicon suggests a 5' boundary for *Gdf6* enhancers. Alternatively, the rearrangement may have separated rodent *Gdf6* from ancestral regulatory elements, or brought novel regulatory sequences into proximity with *Gdf6*, either of which may have had evolutionary consequences for rodent skeletal morphology.

**Evolution of the Alcohol Dehydrogenase Class I Gene Cluster in Human and Non-human Primates.** *H. Oota, W.C. Speed, J.R. Kidd, K.K. Kidd.* Dept Genetics, Yale Univ, New Haven, CT.

The human alcohol dehydrogenase (ADH) gene cluster on chromosome 4 includes seven genes subdivided into five classes based on kinetic properties and sequence similarities. Human Class I ADH genes (*ADH1A*, *ADH1B*, *ADH1C*), expressed in liver, are the main enzymes catalyzing alcohol oxidization to aldehyde in humans. The three Class I genes occur tandemly separated by ~ 15kb and show very high nucleotide similarity both in exons ( 90%) and introns ( 70%) suggesting recent duplication events, probably within the primate lineage since rodents have only one Class I gene. A previous comparative study of exons and 5 and 3 non-coding regions in humans and monkeys concluded that there were at least three gene conversion events in the evolution of Class I ADH genes. Our preliminary analysis of the complete human sequence shows complex similarities among the flanking regions of the genes but no clear indication of the order of gene duplication or of gene conversion. To provide better evolutionary histories, we have sequenced introns 2 and 3 of all three Class I genes (total 7.0 kb) for five non-human primates four apes (chimpanzee, bonobo, gorilla, and orangutan) and one monkey (baboon) and compared the sequences with those of human and mouse. The sequences within each gene cluster largely as expected with baboon as the outgroup, then orangutan, then gorilla, human, chimpanzee, and bonobo. All three genes show similar diversification for both introns. Assuming the baboon divergence at 25 My BP, the diversification between genes is at least another 30 My BP, placing the duplication close to and probably prior to the divergence of the prosimians in the primate lineage. There is little difference in the relative divergences of the three genes and no statistical support for order of duplication using mouse sequence. Thus, at least for these sequences there is no evidence of gene conversion and effective triplication is dated to near the origin of the primate order. [Supported in part by NIH grant AA09379].

**Analysis of Conserved Domain evolution: Conservation does not equal Constraint.** *G.J. Wyckoff<sup>1</sup>, C.M. Malcom<sup>2</sup>, J. Paschall<sup>1</sup>*. 1) Mol. Biol. Biochem., U. Missouri-Kansas City, Kansas City, MO. 64110; 2) Dept. of Anthropology, U. Chicago, Chicago, IL 60637.

Efforts such as the Conserved Domain Database (CDD; <http://www.ncbi.nlm.nih.gov/>), indicate that many structural and functional protein domains are conserved over evolutionary time. In this study, we examine patterns of molecular evolution between orthologous genes across species from the perspective of domain structure. The goal is to determine how sequence conservation within domains translates into functional constraint, and how variation and selective pressure within these otherwise conserved sequences may lead to adaptive protein function. We gathered a set of 6,921 orthologous genes between mice and humans. We aligned the coding regions of these genes in-frame and mapped conserved domain locations on to aligned protein sequences. Using a database we designed to mine sequence and evolutionary information, we generated data for a set of 16,728 Conserved Domain features within these genes. We calculated synonymous divergence rate (Ks), nonsynonymous divergence rate (Ka), and constraint (Ka/Ks) (Li 1993). Results show that, although conserved domains in general possess low enough variation to be detected as conserved, the pattern of variation among them reflects unique evolutionary pressures beyond simple constraint. We find that many conserved domains are the site of elevated Ka rates when scaled by synonymous mutation rate. A Ka/Ks > 1.0 normally implies positive, Darwinian selection. Although domains with a Ka/Ks > 1.0 were infrequent, many had Ka/Ks rates significantly greater than the those of the genes of which they are a part (7% of all domains). This result implies either a lack of functional constraint within these domains, which seems unlikely for a sequence conserved by evolution, or could indicate adaptation within these domains toward a new function. Specifically, we identify domains in genes implicated in human diseases for which the domain Ka/Ks is divergent from the rest of the gene it resides within or in comparison to the same domain type in other genes. We suggest ways to incorporate this type of information into disease gene analysis and mapping.

**Evolutionary analysis of metabolic genes and inborn errors of metabolism.** *J.E. Paschall, V.L. Nelson, G.J. Wyckoff.* Mol. Biol. Biochem., U. Missouri-Kansas City, Kansas City, MO. 64110.

Inborn errors of metabolism (IEMs) offer a unique opportunity to study patterns of evolutionary constraint for disease related genes. We examined orthologous genes between humans and mice that are responsible for IEMs to compare evolutionary constraint at these disease-linked loci to other sets of loci that are either metabolic or non-metabolic. Drawing from our database of 6,921 orthologous genes aligned in-frame between humans and mice, we identified 86 distinct genes responsible for 293 IEMs. We calculated synonymous divergence rate (Ks), nonsynonymous divergence rate (Ka), and functional constraint (Ka/Ks) for the entire set of orthologous genes (Li 1993). Genes causing IEMs were identified based on the SSIEM database (<http://www.ssiem.org.uk/>). This data was combined with our representation of metabolic pathway topology based on KEGG (<http://www.genome.ad.jp/kegg/>) and expression data from Unigene (<http://www.ncbi.nlm.nih.gov/Unigene>). Our hypothesis was that genes causing IEMs are less able to tolerate variation at either the nucleotide or chromosomal level than non-IEM genes; in short, we expected higher levels of constraint to correlate with disease status. Results show that genes involved in general metabolic functions have significantly lower levels of evolutionary constraint (Ka/Ks of approximately 7% lower on average) compared to non-metabolic genes ( $p < .001$  by either F-test or t-test). However, when a set of IEM genes and a set of general metabolic (non-IEM associated) genes are compared, no significant difference in evolutionary constraint is found. Additionally, there is no strong correlation between the number of different IEMs related to a given gene and Ka/Ks. We discuss the use of gene expression, metabolic relationships, and protein domain structure data to devise more sensitive tests of constraint and discuss a database schema used to perform these analyses. Further, we suggest ways in which these observations may be generalized for the examination of Mendelian and complex disease states.

**Compositional Constraints on X Chromosome and XY Gene Pairs Suggest Selection Pressure on Silent Sites. M.**

*Patel<sup>1</sup>, J.S. Sinsheimer<sup>2</sup>, E.R.B. McCabe<sup>3</sup>, A.P. Arnold<sup>4</sup>.* 1) Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Canada; 2) Depts of Biomathematics and Human Genetics; 3) Dept of Pediatrics, David Geffen School of Medicine at UCLA, Dept of Human Genetics and Molecular Biology Institute; 4) Dept of Physiological Science and Laboratory of Neuroendocrinology of the Brain Research Institute, UCLA, Los Angeles CA USA.

We have previously shown that the X-linked gene, NR0B1, encoding the protein DAX1, is predisposed to amino acid-changing substitutions similarly to the Y-linked SRY and greater than the autosomal SOX9. Here, we compare NR0B1 (DAX1) sequence changes during evolution with other X-linked genes and X-Y gene pairs to determine if evolutionary profiles could be correlated with chromosomal location. Genes are thought to reflect the GC content of the isochore in which they reside, particularly in the GC content at the 3rd position of synonymous codons (GC3s). To address whether genes mapped to the same isochore show similar silent site evolution, we compared 573 X-linked genes, covering the entire human X. We found that genes mapped in close physical proximity ( 500 kb) in the same chromosomal band show differences in synonymous codon usage, overall GC and GC3s content; the differences in GC3s were significant enough to assign neighboring genes to different isochores. We then compared X-Y gene pairs (X-linked genes with active Y chromosome homologs). We found that the X copy resembles the Y linked copy more than its X-linked neighbors in GC3s content and synonymous codon usage. We then compared the nucleotide sequence of X-Y gene pairs in human and mouse. The number of non-synonymous substitutions per non-synonymous site (Ka) and synonymous substitutions per synonymous site (Ks) were calculated in pairwise comparisons for genes between species. X genes across species were more similar to each other than the X gene was to its Y copy in the same species. We conclude that gene specific influences preclude silent site homogenization with flanking sequences on the X chromosome, and that for X-Y gene pairs, X chromosome specific forces exert a dominant influence on X genes that results in sequence conservation across species.

**Identification of great ape and human lineage-specific genes using cDNA array-based CGH.** *J. Sikela<sup>1</sup>, K. Marshall<sup>1</sup>, Y. Kim<sup>4</sup>, E. MacLaren<sup>1</sup>, G. Hahn<sup>2</sup>, L. Meltesen<sup>2</sup>, R. Hink<sup>1</sup>, S. Burgers<sup>1</sup>, T. Hernandez-Boussard<sup>5</sup>, A. Karimpour-Fard<sup>3</sup>, D. Glueck<sup>3</sup>, L. McGavrin<sup>2</sup>, R. Berry<sup>2</sup>, J. Pollack<sup>4</sup>.* 1) Human Medical Genetics Program; 2) Clinical Cytogenetics Laboratory; 3) Department of Preventive Medicine, University of Colorado Health Sci. Ctr. Denver CO; 4) Department of Pathology; 5) Department of Genetics, Stanford University, Palo Alto CA.

Gene duplication is thought to be an important mechanism in hominoid evolution and speciation, and in the development of new biological processes and adaptations. While it has been estimated that approximately 5% of the human genome is comprised of segmental duplications, a genome-wide analysis of gene amplification (or loss) has not yet been reported for human or any other hominoid. To identify gene copy number changes unique to specific hominoid lineages, interspecies cDNA array-based comparative genomic hybridization (aCGH) was used to individually compare 39,711 cDNAs, representing 29,648 human genes, across five hominoid species: human, bonobo, chimpanzee, gorilla and orangutan. 1,026 genes were identified that produced hybridization signals unique to one or more of the hominoid lineages, including 135 that were human lineage-specific. Verification of the accuracy of the aCGH data was made by comparison to published estimates of hominoid gene copy numbers for two different genes. Additional confirmation was obtained by cross species FISH analysis using BAC probes containing genes predicted to be amplified only in the human lineage. Using software (Treeview) that allows genes to be visualized in the order in which they occur in the genome, genes showing lineage-specific copy number changes were identified that occurred either as single isolated genes or as clusters of contiguous or nearly contiguous genes. Of 23 lineage-specific gene clusters identified, the majority (17) were positionally biased in the genome, occurring more frequently in particularly dynamic genomic regions. Measured as a function of the evolutionary age of each lineage, gene copy number expansions (129) were most pronounced in human, and include a number of genes thought to be involved in the structure and function of the brain.

**Comparative transcriptome analysis of Hirschsprung versus normal human colon.** *M. Clement-Ziza<sup>1</sup>, J.P. Jaïs<sup>2</sup>, T. Attié-Bittach<sup>1</sup>, J. Augé<sup>1</sup>, F. Jaubert<sup>3</sup>, D. Jan<sup>4</sup>, A. Munnich<sup>1</sup>, S. Lyonnet<sup>1</sup>, G. Pitu<sup>5</sup>, L. Aggerbeck<sup>6</sup>, C. Besmond<sup>1</sup>.* 1) INSERM U393, Hôpital Necker-Enfants-Malades, Paris, France; 2) Biostatistique et Informatique Médicale, Hôpital Necker-Enfants-Malades, Paris, France; 3) Anatomopathologie, Hôpital Necker-Enfants-Malades, Paris, France; 4) Chirurgie pédiatrique, Hôpital Necker-Enfants-Malades, Paris, France; 5) CEA, Evry, France; 6) Centre de Génétique Moléculaire, CNRS, Gif-sur-Yvette, France.

Hirschsprung disease (HSCR), the most common hereditary cause of human intestinal obstruction, 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. RET is the major disease gene and accounts for about 50% of mutations. Mutations in other genes such as GDNF, EDNRB and SOX10 genes fail to explain the transmission of the disease. Recently, a large non-parametric study described HSCR as multigenic disease (Nat Genet. 2002; 31:89-93).

In order to tentatively identify genetic markers of the HSCR colon and other genes involved in the disease, we carried out microarray comparative transcriptome expression studies.

We analysed the transcriptome of HSCR versus normal colon, using cDNA microarrays containing about 4000 human genes. We identified differentially expressed genes in the HSCR context. This led to the determination of specific marker genes for the HSCR phenotype. These genes are also potential candidate genes for a possible involvement in HSCR.

In conclusion, this study helps us to identify candidate genes for HSCR, genetic markers for this disease and should lead to a better understanding of the physiopathology of the gut function. Further studies will include microdissection steps in order to investigate the transcriptome of the Meissner and Auerbach plexi.

**Time-Resolved fluorometric DNA hybridization assay for the determination of celiac disease associated HLA alleles.** *H. Grey, M. Sjoroos, H. Sahlberg.* Life and Analytical Sciences, PerkinElmer, Turku, Finland.

Genetic susceptibility to celiac disease is strongly associated with HLA-DQA1\*05 - DQB1\*02 (DQ2 heterodimer) and HLA-DQA1\*03 - DQB1\*0302 (DQ8). The primary importance of HLA-DQ alleles conferring the disease susceptibility and the rarity of patients carrying neither the DQ2 nor DQ8 heterodimers have been frequently emphasized. The HLA typing assay developed relies on the amplification of the exon 2 fragments of DQA1 and DQB1 genes as well as solution hybridization with lanthanide-labeled allele-specific oligonucleotides. Hybridization reactions are measured in microtitre plate format using time-resolved fluorometry detection. The temporal and spectral resolution in time-resolved fluorometry allows the measurement of three different labels (europium, samarium and terbium) in the same well. For the interpretation of the results, a MultiCalc program is used to calculate the signal-to-background ratios and normalize the difference in amplification levels of individual samples. Both inter- and intra-assay as well as total variation (% CV) was found to be < 20 (10 PCR products in 10 runs with 2 replicates per plate). To validate the specificity of the developed assay, selected samples were genotyped using sequencing as a reference method. A correct classification of the genotypes confirmed the accuracy of our HLA assay. The developed assay is fast, specific, easy-to-perform and ideally suited for HLA typing because of the possibility to use multiple lanthanide chelates to label the probes. In addition to exploiting isolated genomic DNA as PCR template, the assay also supports the direct use of dried whole blood spots without any sample pre-treatment steps.

**Characterisation of the gene expression profile of human fetal cartilage.** *R. Pogue<sup>1</sup>, E. Sebald<sup>1</sup>, L. King<sup>1</sup>, A. Day<sup>2</sup>, F.E. Castro<sup>2</sup>, E. Kronstadt<sup>1</sup>, S. Nelson<sup>2</sup>, D. Krakow<sup>1</sup>, D. Cohn<sup>1</sup>.* 1) Dept Pediatrics, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) Department of Human Genetics, University of California, Los Angeles.

The transcriptional profile of human cartilage, and fetal cartilage in particular, remains poorly characterised in the databases of the human genome project. To address this discrepancy, and to identify genes whose expression is restricted to cartilage, we constructed a cartilage cDNA library derived from the humeri and femurs of two 18-20 week old human fetuses. Sequence data were obtained for 6,266 individual clones, and the genes represented by these were identified by BLAST analysis. Our analysis of the most frequently represented genes in the library indicated that 178 genes accounted for 47.1% of the transcripts in the tissue. Of these, 44 were not previously known to be expressed in cartilage. The majority of the genes had been previously characterised in terms of function, sub-cellular localisation and/or homology to known genes. These included a number of genes known to be crucial to cartilage function by virtue of the fact that mutations in them give rise to osteochondrodysplasias, diseases of skeletal development and growth. The library also contained approximately 600-700 uncharacterised genes, genes for which the sequence is known but the function is unknown. Fourteen of the 178 most highly expressed genes were uncharacterised. Affymetrix microarray analysis, with confirmation by real-time PCR, was used to determine the tissue expression profiles of the genes, showing that 29 of the top 178 genes, including 3 uncharacterised genes, are cartilage specific. We present here a complete summary of the transcriptional profile of 18-20 week human fetal cartilage, including expression data identifying those genes with a cartilage-specific profile. The genes in the library represent potential candidates for the roughly 170 forms of osteochondrodysplasia for which the causative gene has not yet been identified.

Program Nr: 1534 from 2003 ASHG Annual Meeting

**Polymorphism of A-G at canine mtDNA 2683 corresponding to human mtDNA A3243G mutation.** *F.Y. Li, I.D. Duncan.* Dept Medical Sci, Sch Vet Med, Univ Wisconsin, Madison, Madison, WI.

Many efforts have been made to generate animal models with mtDNA mutations but only a few have succeeded. We screened the canine mtDNA encoded tRNA<sup>Leu</sup>(UUR) gene in 26 domestic dogs and found 16 dogs have a G while 10 have an A at canine mtDNA 2683, corresponding to human mtDNA A3243G mutation. The result was further confirmed by Southern blot analysis digested with Apa I and by Apa I digestion of nest PCR products. This result provides direct evidence that pathogenic mtDNA mutation in humans could be non-pathogenic in animals, suggesting that caution should be taken before attempts are made to generate an animal model with mtDNA mutations.

Program Nr: 1535 from 2003 ASHG Annual Meeting

**Development of Resequencing Primer Sets for High Throughput DNA Sequence Variation Studies.** *B. Nutter, J. Sorenson, C. Gire, M. Rydland, P. Shen, S. Glanowski, N. Edwards, M. Furtado, K. Guegler, E. Spier, R. Fang, L-Z. Pham.* Applied Biosystems, Foster City, CA.

The completion of a reference sequence for the human genome and improvements in high-throughput sequencing technology, including the Applied Biosystems 3730xl DNA analyzer and the BigDye Terminators v3.1 Cycle Sequencing Kit, have motivated the development of easier solutions for quickly resequencing human genes. Designing primers for robust PCR amplification of specific regions of the genome continues to present a significant challenge to high throughput resequencing studies. We report here results of our development of a validated process for designing primers for high-throughput amplification and resequencing of the promoter regions, exon regions, and flanking intronic regions for genes implicated in cancer and other diseases. Primer design for large scale resequencing projects has been greatly improved by our ability to correlate both unsuccessful PCR amplification and poor quality sequencing results to the presence of local and global factors in the genome. Using very large datasets of PCR primer amplification results (>100,000 amplicons) and sequencing data (>1,000,000 sequence files) generated during the Applera Genome Initiative, we are developing a model that will be predictive of the success rate for a given amplicon. We will present the results of a comparison of the success of primer amplification and generation of high quality sequence data in the laboratory to the success predicted by our model. The validation of a model for highly successful PCR primer design will permit the resequencing of all genes from a number of genomes.

**Variation in gene expression in lymphoblastoid cell lines.** *T. Rosser, F. Zhang, S.T. Warren.* Dept Human Genetics, Emory Univ, Atlanta, GA.

Gene chip microarrays are a now routine way to identify changes in gene expression for a variety of diseases and disorders. Often the amount of information gained from these analyses is overwhelming and must be carefully examined to determine significant results. We have investigated the variation in gene expression in lymphoblastoid cell lines through the use of Affymetrix U133 A and B gene chip microarrays in order to reduce the number of false positives to more quickly identify relevant results. We evaluated RNA samples from control cell lines created from three separate blood draws from two individuals, F (female) and M (male), as well as RNA samples extracted from the same cell line, F, at different time points in culture. We were able to show that there is variation between normal cell lines as expected but that there is also variation among cell lines created from the same individual. When we compared all 3 cell lines for F we saw 149 probe sets changed on the A chip with 18 above 1 signal log ratio (SLR) and only 1 above a 2 SLR. For the B chip, we observed 55 probe sets changed with only 4 above a 1 SLR and 0 above a 2 SLR. When we compared all three of the M cell lines, we saw 230 probe sets changed for the A chip with 21 above a 1 SLR and 0 above a 2 SLR. A comparison of the 2 cell lines with a pool of 5 control male cell lines showed an average of 2098 probe sets changed for F U133A, 1268 for F U133B, 2745 for M U133A and 2088 for M U133B. Of these only 49 were shared changes between F and M. As expected, the expression of several Y linked genes was not detected in the F cell lines. When we compared the 3 RNA samples from the F cell line at different culture time points, we observed 48 probe sets changed for the A chip with only 1 above a 1 SLR and only 12 changed for the B chip with none above a SLR of 1. We were also able to compile a set of probe sets that are reproducibly variable between controls and can serve as a mask when attempting to identify true gene expression changes if using lymphoblastoid cell lines as your RNA source.

**An Integrated Haplotype Map of the Human Major Histocompatibility Complex.** *J.D. Rioux<sup>1</sup>, K. Mather<sup>2</sup>, S. Schaffner<sup>1</sup>, L. Farwell<sup>1</sup>, M. Daly<sup>1</sup>, N. Patterson<sup>1</sup>, M. Cullen<sup>3</sup>, M. Carrington<sup>3</sup>, T. Bugawan<sup>4</sup>, H. Erlich<sup>4</sup>, J. Campbell<sup>1</sup>, J. Barrett<sup>1</sup>, K. Miller<sup>1</sup>, G. Thomson<sup>2</sup>, E.S. Lander<sup>1</sup>, E.C. Walsh<sup>1</sup>.* 1) Center Genome Research, Whitehead Inst, MIT, Cambridge, MA; 2) Dept. of Integrative Biology, University of California, Berkeley, CA; 3) Basic Research Program, SAIC-Frederick, Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD; 4) Roche Molecular System, Department of Human Genetics, Alameda, CA.

Numerous studies have clearly indicated a role for the Major Histocompatibility Complex (MHC) in susceptibility to autoimmune diseases. Such studies have focused on the genetic variation of a small number of classical human leukocyte antigen (HLA) genes in the region. Although these genes represent good candidates given their immunological roles, linkage disequilibrium (LD) surrounding these genes has made it difficult to rule out neighboring genes, many with immune function, as influencing disease susceptibility. It is likely that a comprehensive analysis of the patterns of LD and variation using a high density of single nucleotide polymorphisms (SNPs) would enable a greater understanding of the nature of the observed associations as well as lead to the identification of causal variation. We present herein an initial analysis of this region using 201 SNPs, 9 classical HLA loci, 2 TAP genes, and 18 microsatellites. This analysis suggests that LD and variation in the MHC aside from the classical HLA loci are essentially no different than that in the rest of the genome. Furthermore, we also demonstrate that multi-block SNP haplotypes contain considerable predictive information for common HLA alleles. An additional implication of these results is that multi-block SNP haplotypes may be sufficient to identify low frequency variants throughout the genome. Such low frequency variants would likely be missed in single block-based common variant analysis, however their contribution to disease could be assayed by using multi-block haplotypes in analysis. Finally, even though this map is not yet complete, the data presented will show that multi-SNP haplotypes will likely be a valuable means for refining disease association signals in this region.

**Detailed annotation and search for disease causing genes on 8q22-qter.** *S. Asakawa<sup>1</sup>, A. Shimizu<sup>1</sup>, S. Yamazaki<sup>1</sup>, S.K. Ishikawa<sup>1</sup>, T. Sasaki<sup>1</sup>, J. Kudoh<sup>1</sup>, S. Minoshima<sup>1</sup>, I. Kondo<sup>2</sup>, N. Shimizu<sup>1</sup>.* 1) Dept Mol Biol, Keio Univ Sch Med, Tokyo, Japan; 2) Dept Hyg, Ehime Univ Sch Med, Ehime, Japan.

We have finished sequencing of 180 BAC clones covering 20 Mb of human chromosome 8q22-q24.1. Furthermore, we have made detailed annotation of more than 55 Mb of genomic sequence on 8q22-qter by homology search, exon prediction and calculation of GC/CpG content. The homology search by BLAST was performed against nr, est database and Fugu whole genome shotgun sequences. For exon prediction, several prediction programs such as GENSCAN, X-Grail and MZEF were employed. All of the data obtained by these computational analyses were integrated using spreadsheet software, and then visualized graphically by a macro-program developed on a CAD program. More than 250 protein-coding genes were identified in the 8q22-qter. Computer-predicted proteins from many of those genes possessed no obvious domains and motifs, and hence we designated those proteins as Kao-nashi. We also calculated the gene density and found a gene poor region (1 gene / 6 Mb) and a gene rich region (24 genes / 250 kb). On 8q22-qter, there are several disease genes mapped, including a familial epilepsy BAFME/FAME. The BAFME/FAME is an autosomal dominant idiopathic epileptic syndrome characterized by adult-onset tremulous finger movement, myoclonus, epileptic seizures, and a nonprogressive course, recognized mainly in Japanese families. Although the disease locus was assigned to 8q23.3-q24.1, the causative gene has not been identified. We listed more than 40 candidate genes within the disease locus based on our detailed gene map of 8q23.3-q24.1. We are now doing mutation analysis of these genes using DNA from BAFME patients.

Program Nr: 1539 from 2003 ASHG Annual Meeting

**Statistical Inference of Haplotype Reconstruction From SNP Alignment.** *J. Kim*<sup>1</sup>, *L. Li*<sup>2</sup>, *M.S. Waterman*<sup>2</sup>. 1) Computer Science, USC, Los Angeles, CA; 2) Computational Biology, USC, Los Angeles, CA 90089.

The goal of shotgun sequencing is to reconstruct the underlying target chromosome from random fragments. It usually consists of two steps: alignment that results in an assembly matrix; construction of consensus. In some sequencing projects, the targets contains two homologous chromosomes and our aim is to distinguish between polymorphisms and sequencing errors. The difficulty lies in the fact that origins of fragments are unknown. Li, Kim and Waterman proposed a method of haplotype reconstruction from SNP Alignment. It is based on a probabilistic model that includes sequencing error rates, compositional information of nucleotides, and haplotype frequency. The method assumes that the parameters are known. For example, the chance of one fragment being generated from one parental chromosome is assumed to be half. In practice, these parameters need to be estimated. We develop inference procedures for haplotype reconstruction. First we obtain a fast algorithm to calculate the likelihood of the observed assembly matrix as a function of haplotype frequency. It is based on an interesting Markov structure along fragment alignment. The complexity of the algorithm is linear with respect to the number of SNP loci. Consequently, this allows us to find the maximum likelihood estimate of the haplotype frequency. We explain an irregular phenomenon due to the the complicated data structure of shot sequencing. To estimate other parameters, we develop an EM algorithm. In addition, we can take the quality values such as Phred scores into consideration. We will present our simulation results with different settings of parameters. Reference: 1. Churchill, G.A., and Waterman, MS. The accuracy of DNA sequences: Estimating sequence quality. *Genomics*, 14:89-98, 1992. 2. Li, L, Kim JH, Waterman MS. (2003). Haplotype reconstruction from SNP alignment. RECOMB03.

**Towards a Complete Transcript Map of Human Chromosome 22: Comprehensive Gene Search and Functional Analysis of Novel Genes.** S. Minoshima, T. Sasaki, A. Shiohama, K. Hosono, H. Yang, T. Izumiyama, S. Asakawa, J. Kudoh, N. Shimizu. Dept Molecular Biol, Keio Univ Sch Medicine, Tokyo, Japan.

We built the first milestone of human genome research that is the completion of chromosome 22 sequencing (*Nature* **402**: 489-495, 1999). In the report, we identified 545 genes with semi-automatic computational procedure. Since then, we have been doing extensive manual annotation and experimental isolation of cDNAs to draw a complete transcript map substantiated with information of all exons, alternative transcription, expression profile and function for each gene.

To date, we finished analyzing the proximal 40% of 22q (14 Mb), which includes the Cat Eye Syndrome (CES) region, DiGeorge Syndrome (DGS) region, IGL-IGLL region, and CRYBB region, and identified 15 new genes. Of these, we report here the initial characterization of 2 genes, DGCR8 and PIWIL3. DGCR8 is located in the DGS region. Northern blot analysis revealed that DGCR8 is transcribed in three forms, and expressed ubiquitously in various organs/tissues of both human and mouse origins. We isolated a cDNA of mouse homolog Dgcr8 that has 95.3% amino acid identities with human DGCR8. The protein domain analysis revealed one WW motif and 2 double stranded RNA binding motifs. Expression patterns of developing mouse embryo indicated this gene appears to be associated with some clinical phenotypes of DGS (*BBRC* **304**: 184-190, 2003). Further analyses on the DGCR8 protein function and phenotype-gene copy number relation are in progress. PIWIL3 is a novel member of the Argonaute family locating 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 that are considered as a common architecture of the Argonaute family. Since PIWIL3 associated with DICER, we postulated it might be involved in the mechanism of RNAi (*Genomics*, in press). Further studies on the interaction of RNA with PIWIL3/DICER proteins are in progress. We will also present the data on the remaining 13 new genes (CESK1, GAB2L, DGCRK8, DGCRK9, LUK1, C22orf13, ZNF70, C22orf15, C22orf16, SLC2A11, C22orf14, LLN12 and LLN14).

**A model of Measurement Error for Shotgun Sequencing.** *M. Li, L. Li.* Mathematics, Univ. of Southern California, Los Angeles, CA.

In shotgun sequencing, statistical reconstruction of the consensus from alignment requires a model of measurement errors. Churchill and Waterman proposed such a model and an EM algorithm to estimate these sequencing error rates from assembly matrix. From another perspective, Ewing and Green defined quality scores for base-calling from sequencing traces in such a way that it has a probabilistic interpretation. To achieve the goal, the training of the model that defines the Phred scores has to take a large amount of data. However, in practice sample preparations and sequencing machines may work under different conditions and thus quality scores need to be adjusted. Also the information given by quality scores are incomplete in the sense that they evaluate chances of base-calling errors but they do not tell us the error pattern. We do observe that each nucleotide base has its own specific error patterns. We develop models of measurement error for shotgun sequencing by combining the above two perspectives. One option is to represent the error pattern of each nucleotide by a multinomial model. Since the true sequence is unknown, we develop an EM algorithm to deal with missing data. In a more sophisticated way, we propose a model with logistic parameterization taking quality scores as covariate. To represent the nonlinear effect of quality scores, we adopt smooth spline models or simple piecewise linear functions in the regression. We apply the method to an assembly of a BAC, and more than 70% errors from the majority rule are corrected.

**DEVELOPMENT OF A RE-SEQUENCING PIPE-LINE FOR HIGH-THROUGHPUT SEQUENCING OF CANDIDATE DISEASE GENES.** *I. Yakub, D. Burgess, A. Goldman, H. Arredondo, D. Khurna, H. Dinh, B. Patel, D. Muzny, J. Noebels, R. Gibbs.* Baylor College of Medicine, Houston, TX.

In order to better understand complex disorders caused by multiple genes with genetic heterogeneity and lack of penetrance, genome wide methods that allow us to screen for mutations in multiple loci in a large sample set need to be developed. We have established a pipeline to re-sequence large numbers of candidate disease genes in several individuals in a high-throughput manner. A batch primer design software is being used to design primers to amplify all coding regions of every candidate disease gene. All primers also contain a 20mer universal forward or reverse sequencing primer tail. PCR is carried out at a standardized condition and the products are purified. The purified products are then sequenced using the universal primers and the sequences are analyzed for SNPs using a mutation detection software. Several of these steps have been automated allowing re-sequencing to be carried out in a high throughput manner with increased efficiency and lower cost. This pipeline is being used to re-sequence 208 channelopathy genes, several of which have been associated with various forms of epilepsy. In general, epilepsy shows a complex mode of inheritance, although occasionally, a mendelian pattern of inheritance is observed. Epilepsies are grouped into 3 different sub-classes known as idiopathic, symptomatic and cryptogenic. Several epilepsy genes have been identified for each type that elevates the risk of the disorder in individuals carrying variant alleles. This re-sequencing effort is being carried out in 46 epileptic individuals and 46 unaffected controls.

**Comprehensive analysis of conserved non-genic sequences (CNGs) on human chromosome 21.** *E.T. Dermitzakis<sup>1</sup>, A. Reymond<sup>1</sup>, C. Ucla<sup>1</sup>, E. Kirkness<sup>2</sup>, L. Excoffier<sup>3</sup>, S.E. Antonarakis<sup>1</sup>*. 1) Div Medical Genetics, Univ Geneva, Geneva, Switzerland; 2) The Institute of Genome Research, Rockville, Maryland, USA; 3) Zoological Institute, University of Bern, Bern. Switzerland.

The recent comparison of the human genome and mouse genomes brought to light a large number of conserved non-genic sequences (CNGs) (Dermitzakis et al. 2002, Nature 420: 578, Waterston et al. 2002, Nature 420: 520). Although their conservation is high enough to strongly support their functionality, the actual role of these sequences remains unknown. We have performed a comprehensive analysis of CNGs on human chromosome 21 (Hsa21) looking at i) sequence conservation across multiple mammalian species including a marsupial and a monotreme, ii) sequence comparison with a 1X dog genome shotgun, and iii) patterns of polymorphism within a sample of 10 French CEPH individuals and 10 West Africans. The comparison of 220 of the CNGs across 14 mammalian species showed very high conservation and distinct characteristics and lower rate of evolution from coding and ncRNA genes. In addition there appears to be subcategorization within CNGs suggesting the presence of different functional classes. Comparison of 1628 CNGs and 950 conserved exons of Hsa21 between human, mouse, and dog reveals that selective constraint does not depend on the position on the chromosome or proximity to genes and suggests a different role more global than the regulation of genes in cis. Finally, analysis of variation of 95 CNGs and comparison with exons, introns, regulatory regions and random intergenic sequences on Hsa21 within humans suggests that the levels of polymorphism, although lower than the average of the genome, is higher than the one observed in exons, which suggests that there could be considerable genetic variation harbored in these regions. We conclude that many of the CNGs have functional characteristics that are unique and probably constitute a new class of functional genomic elements with only partial similarity to already known ones. Our data also suggest that CNGs are good targets for functional variation in human populations and mutation searches for phenotypic consequences should focus on such regions as well.

**Whole Genome BAC Microarrays: Development and Applications.** *W.W. Cai<sup>1</sup>, J. Li<sup>1</sup>, T. Jiang<sup>1</sup>, E. Rajcan-Separovic<sup>2</sup>, Q. Li<sup>1</sup>, B. Bejjani<sup>3</sup>, S.W. Cheung<sup>1</sup>.* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030; 2) Department of Pathology, University of British Columbia, 4480 Oak Street, Vancouver, V6H 3V4, British Columbia; 3) Basic Medical Sciences Program, Washington State University Spokane Health Research and Education Center, P.O. Box 1495, Spokane, WA 99210-1495.

Chromosomal Deletions and duplications are common genomic abnormalities in a wide range of human syndromes. It has long been suspected that a substantial proportion of many human developmental deficiencies currently classified as idiopathic might be caused by small genomic rearrangement beyond the resolution of current technologies. To enable detection of such subtle rearrangements, we have developed DNA array-based whole genome (WG) BAC arrays that can scan the genome at a resolution of exceeding 200kb. Our human WG arrays contain 21,500 sequenced BAC/PAC clones, which covers the human genome at an average resolution of 7 clone/MB, with virtually all regions with sufficient unique sequences covered in contigs. We validated the human WG arrays blindly using a set of samples with known chromosome abnormalities. We have applied the HWG arrays to analyze samples from families in which the child is mentally retarded but the parents have normal IQs. Karyotypic analysis of these families did not reveal any chromosomal abnormalities. However, HW array analysis of these patients samples indicated that there were a considerable number of chromosomal rearrangements of small size, with most of them localized in the subtelomeric regions. Most of the abnormalities were de novo. Some of the abnormalities were consistent between different families. We also detected consistent rearrangements in patients as well as both parents. These results corroborate the hypothesis that some of the idiopathic syndromes indeed have a genetic basis. We also developed WG BAC arrays to cover the entire mouse genome in contigs. The mouse arrays have been extensively used to analyze chromosomal abnormalities in mouse tumors and to detect genomic imbalance polymorphisms between inbred mouse strains.

**EXO-Free SNP Genotyping Assay Based On Fluorescence Polarization Detection.** *S. Duan*<sup>1</sup>, *P. Taillon-Miller*<sup>1</sup>, *R. Miller*<sup>1</sup>, *P-Y. Kwok*<sup>2</sup>. 1) Division of Dermatology, Washington University, St. Louis, MO 63110; 2) Cardiovascular Research Institute and Department of Dermatology, University of California, San Francisco, CA 94143.

Template-directed dye-terminator incorporation with fluorescence polarization (FP-TDI) detection assay is a widely used homogeneous SNP (single nucleotide polymorphism) genotyping method. The assay basically includes 4 steps: 1) PCR reaction; 2) PCR product clean up with *E. coli* exonuclease I (EXO) and shrimp alkaline phosphatase (SAP) to degrade the excess PCR primers and dNTPs; 3) single base primer extension; and 4) FP reading. In further development of this assay, we have eliminated the EXO degradation part of the PCR clean-up step by designing PCR primers so that the base immediately 3' to each primer is different from the polymorphic bases of the SNP. With this design, the PCR primers will not interfere with the primer extension step of the assay. The simplified PCR clean-up step therefore requires only degradation of dNTPs with SAP. For example, the PCR primers for a SNP with C/T alleles will be designed such that the bases 3' to the primers will only be A or G (but not C or T). In this assay, the excess PCR primers in the primer extension step of the assay will not get extended. Assays for 50 SNP markers based on this design principle were genotyped in both directions and the results were in strict concordance with previously determined results. Elimination of EXO in the FP-TDI assay further reduces the cost of this simple SNP genotyping method.

**High throughput assay identifying a LINE insertion to confirm species ID in humans.** *P. Bender<sup>1</sup>, J. Beck<sup>1</sup>, D. Bartnik<sup>1</sup>, K. Smith<sup>1</sup>, L. Mathews<sup>2</sup>, G. Swergold<sup>2</sup>*. 1) Coriell Inst Medical Research, Camden, NJ; 2) Department of Medicine, Columbia University, New York, NY.

The Coriell Cell Repositories distribute both human and non-human primate DNA and cell lines. Any laboratory environment that works with biomaterials from more than one species needs a high-throughput, cost effective assay to verify species. We have developed a PCR-fluorogenic assay for identifying human DNA using unique properties of LINEs (long interspersed nuclear elements). LINEs are retrotransposable elements, many of which are no longer actively amplifying and transposing. The youngest LINEs in the human genome (L1) are useful for species identification because they inserted soon after the emergence of the human lineage. Forward and reverse primers were designed bordering a L1 insertion that amplify a 6.4 kb L1 insert unique to humans. In all other non-human hominoid species, an "empty" band of <1kb is amplified from the conserved sequences that border the insertion site. We tested over 80 individuals representing diverse ethnic origins such as African American, Chinese American, European American, and Mexican American. All samples amplified the expected fragment of 6.4 kb and no sample amplified the "empty" band. These results support the claim that this L1 insertion is fixed in humans. The assay was modified to create a PCR-fluorogenic assay, by designing a new reverse primer that anneals to a L1 sequence approximately 340 bp downstream from the forward primer. These primers direct amplification of the 5 L1 insertion site. A fluorogenic probe was designed with a FAM reporter and TAMARA quencher. The probe is complementary to the 8 locus-specific bases bordering the insertion and the first 13 bases of the L1 insertion. This probe is predicted to anneal only at the locus-specific insertion site. The fluorogenic assay generates fluorescence in all 80 human samples tested and no fluorescence was generated from over 25 chimpanzee samples tested. This new method for identifying species-specific LINE insertions is a specific, high throughput, and economical assay for confirming species identification.

Program Nr: 1547 from 2003 ASHG Annual Meeting

**Shape Encoded Particles For Biological Probes.** *Z. Chen<sup>1</sup>, J. Tsai<sup>2</sup>, B. Merriman<sup>1</sup>, J. Chen<sup>1</sup>, C. Kim<sup>2</sup>, S. Nelson<sup>1</sup>.* 1) Dept of Human Genetics, Univ California, Los Angeles, CA; 2) Dept of Mechanical and Aerospace Engineering, University of California, Los Angeles, CA.

We have developed a new system of encoding micro-particles to be used as probe-carriers in massively-multiplexed biological assays. Particle identity is encoded by its shape, and this provides the means to track which binding-reaction probe is coupled to its surface. The particles are fabricated as ~100x100x10 micron square silicon particles cut from wafers using standard Micro-Electro-Mechanical Systems (MEMS) technology. The different shape types are created by unique patterns of 20 notches etched along the edge of the square, which allows production of millions of distinct Shape Encoded Particles(SEP). To apply the system, each distinct probe (e.g., oligonucleotide) is permanently coupled to a corresponding unique SEP in a separate reaction. After the attachment, the different types of SEPs are mixed. A small portion of the mixture, containing representatives of every type of probe-particle, is used in a hybridization assay of a labeled sample. Shape decoding information and probe signal intensities are obtained simultaneously by imaging the particles spread out on a flat surface. Immediate applications of this technology include SNP genotyping and gene expression profiling. We demonstrate the system applied to a SNP genotyping assay. Oligonucleotide tags were attached to the shape encoded particles and specific hybridization was achieved with complimentary dye labeled oligos generated by a Single-Base-Extension reaction. We have also developed analysis software that reads the scanned images, decodes the shapes, computes the fluorescent signal intensities and displays the results.

Program Nr: 1548 from 2003 ASHG Annual Meeting

**Solving The Problem of Limited DNA samples; Comprehensive Whole Genome Amplification Using Multiple Displacement Amplification.** *R.S. Lasken, S. Hosono, C. Bornarth, A. Raghunathan, M. Wisniewski, O. Alsmadi, X. Pan, M. Egholm.* Molecular Staging Inc., New Haven, CT, CT.

Recently we described a new method for whole genome amplification by multiple strand displacement amplification (MDA)(Proc. Nat. Acad. Sci, USA. 99(8), pp. 5261-5266 (2002); Gen. Res. 13:954-964 (2003)). Because of the high level of amplification the procedure can be carried out directly on minute amounts of gDNA, lysed blood, frozen buffy coats, buccal cells or cells from culture generating assay ready DNA for genetic testing. For small samples like laser microdissected cells and needle aspirates, and where large quantities of DNA are required for testing of many SNPs, MDA promises to change the paradigm of genetic testing. A rapid method is demonstrated for bypassing laborious sample preparation steps with DNA amplified from cells or blood being added directly to genetic assays. A high fidelity DNA polymerase assures preservation of correct sequence. Accurate genotyping free of heterozygote dropout results from remarkably unbiased amplification of alleles.

**Use of a three-color microarray platform to control support-bound probe for improved data quality and enable automated image analysis.** *M.J. Hessner<sup>1</sup>, L. Meyer<sup>1</sup>, S. Khan<sup>2</sup>, H. Jacob<sup>2</sup>, S. Ghosh<sup>1</sup>, X. Wang<sup>1</sup>.* 1) Dept Pediatrics, Medical College of Wisconsin, Milwaukee, WI; 2) Dept Physiology, Medical College of Wisconsin, Milwaukee, WI.

DNA microarray fabrication methods have lacked the means to assess quality for all arrays prior to hybridization, thus leaving the array an uncontrolled experimental variable. We have developed a three-color cDNA array platform where printed probes are tagged with fluorescein, which is compatible with Cy3 and Cy5 target labeling dyes when using confocal laser scanners possessing narrow bandwidths. Previously, we have used this three-color approach to 1) develop a tracking system to monitor the printing of probe plates at predicted coordinates; 2) define the quantity of immobilized probe necessary for quality hybridized array data to establish prehybridization array selection criteria; and 3) investigate factors that influence probe availability for hybridization. Recently, we have examined hybridized data filtering using element fluorescein intensity, ratio compression as a function of support-bound probe, and the feasibility of using the third dye image, which possesses less noise and fewer artifacts, for automated processing of the Cy3 and Cy5 images. A direct and significant relationship ( $R^2=0.73$ ,  $p<0.001$ ) between prehybridization average fluorescein intensity and subsequent hybridized replicate consistency was observed, illustrating that data quality can be improved by selecting arrays that meet defined prehybridization criteria. Using printed control probes and transcripts at defined input ratios, we found output ratio compressions of 57% +/-24% versus 81% +/-15% when immobilized probe levels were <5,000 and >10,000 RFU/pixel (fluorescein), respectively. Using these data to establish thresholds, we show our three-color approach provides a means to filter spots from hybridized data sets that possess insufficient bound probe for improved data quality. Lastly, initial results indicate that automated image processing may be considerably enhanced through the use of the third dye image. Collectively, this strategy will improve microarray data and increase its utility as a sensitive screening tool.

**Evaluation of Whole Genome Amplification Methods.** *K.A. Haque<sup>1</sup>, M.B. Beerman<sup>1</sup>, V. Puri<sup>1</sup>, S. Yadavalli<sup>1</sup>, A.T. Crenshaw<sup>1</sup>, R.A. Welch<sup>1</sup>, M. Yeager<sup>1</sup>, B.R. Packer<sup>1</sup>, M. Garcia-Closas<sup>2</sup>, N. Rothman<sup>3</sup>, S.J. Chanock<sup>4</sup>, A.W. Bergen<sup>4</sup>.* 1) Core Genotyping Facility, SAIC-Frederick, Inc., NCI/NIH, Gaithersburg, MD; 2) HREB, DCEG, NCI, NIH, Bethesda, MD; 3) OEB, DCEG, NCI, NIH, DHHS, Bethesda, MD; 4) Core Genotyping Facility, NCI, NIH, Bethesda, MD.

The promise of whole genome amplification (WGA) is that genomic DNA (gDNA) does not need to be the limiting factor during the design and implementation of molecular genetic studies. Two new methods to perform WGA on gDNA have been presented: one method uses the highly processive Phi29 bacteriophage DNA Polymerase with random hexamers by a multiple strand displacement mechanism, and the other method uses chemical fragmentation, linker ligation, and PCR. Validation of three WGA protocols [GenomiPhi DNA Amplification Kit (AB), Amersham Biosciences; REPLI-g 2500S Whole Genome Amplification Kit (MS), Molecular Staging; and OmniPlex (RG), Rubicon Genomics] was performed in the winter of 2002 using the recommended protocols to prepare WGA DNA (wgaDNA) to evaluate concordance of molecular genetic assay results between gDNA and wgaDNA. DNA samples (N=60) extracted from three tissue types were subjected to WGA and quantified to establish wgaDNA yield. DNA yield is dependent on WGA method and is in the range of 6-40 ug (Rank Order ABRGMS), and the CVs were in the range of 9-14% (RGMSAB). Discordance between replicate analyses of gDNA and wgaDNA was assessed through STR (N=9), SNP (N=49 TaqMan), and re-sequencing (N=23 amplicons) assays. STR genotype discordance rates are ~8-12% (ABRGMS) vs. 0.5% using a minimum allelic ratio threshold of 40%. SNP genotype discordance rates are 1% (RGABMS) vs. 0%. SNP undetermined rates are 3-7% (ABMSRG) vs. 1%. SNP non-amplification rates are similar, ~0.4%. SNP completion rates are 92-96% vs. 98% (RGMSAB). Sequence discordance rates will be evaluated comparing quality scores obtained from PhredPhrap. There are differences with respect to overall genotype failure rates among the three methods, where most of the discordant and undetermined genotypes are due to allelic ratio changes and decreased genotype clustering for STR and SNP assays, respectively.

**Robust Dosage-PCR for Detection of Heterozygous Chromosomal Deletions.** *V.Q. Nguyen, J. Shi, X. Li, J.S. Chen, Q. Liu, S.S. Sommer.* Dept Molecular Genetics, City of Hope Medical Ctr, Duarte, CA.

Robust dosage-PCR (RD-PCR) was developed to detect heterozygous large deletions, an important class of mutations missed by conventional PCR strategies. PCR-based methods are available for distinguishing between the dosage of one or two template copies, but general application is limited by the laborious nature of the method and/or the optimization required for each new set of gene exons to be analyzed. RD-PCR depends on a combination of (i) co-amplification of an autosomal and an X-chromosomal segment so that internal dosage controls is integral for any segment to be analyzed and (ii) a robust primer design that includes a 5' tail and a 3' sequence-specific regions in the PCR protocol. The ratio of yields (ROY) of the target to the internal control segment is directly proportional to the ratio of the two input templates over a wide range (at least 1:1 to 1:258 with a correlation coefficient of 0.99). The ROY is not dependent on the amount of genomic DNA or the number of cycles of amplification under typical conditions. RD-PCR eliminates errors in the preparation and manipulation steps by using an endogenous internal dosage control. A blinded analysis of gene dosage was performed to detect deletions of the human factor IX gene with 100% accuracy. RD-PCR assays are presently being utilized to detect chromosomal deletions, insertions, and/or duplications in Hemophilia A and Hereditary Nonpolyposis Colorectal Cancer (HNPCC) patients and family members.

Program Nr: 1552 from 2003 ASHG Annual Meeting

**Detection of extremely rare alleles by bidirectional- pyrophosphorolysis activated polymerization allele-specific amplification (Bi-PAP-A): towards measurement of mutation load in mammalian tissues.** *Q. Liu, S. Sommer.* Dept Molecular Genetics, City of Hope Natl Medical Ctr, Duarte, CA.

A method of detecting one single nucleotide mutation in  $10^6$ - $10^9$  wild type allele would be advantageous for multiple applications including: i) detection of minimal residual disease, especially in solid tumors, ii) cancer risk assessment by searching for elevated mutation load due to endogenous DNA repair defects or environmental mutagen exposures (i.e., measuring the frequency and pattern of somatic mutations present in normal tissues), and iii) prenatal diagnosis of paternally derived mutations within fetal cells in the maternal circulation. Pyrophosphorolysis activated polymerization (PAP) has been developed to detect extremely rare mutations in complex genomes. In theory, PAP can detect a single mutation present in  $3 \times 10^{11}$  copies of wild type alleles. In practice, the selectivity of detection is limited by polymerase extension errors, a bypass reaction, from the unblocked oligonucleotide. Bi-directional PAP allele-specific amplification (Bi-PAP-A) is a novel method that uses two opposing pyrophosphorolysis activatable oligonucleotides ( $P^*$ ) with one nucleotide overlap at their 3' termini, thus eliminates the problematic bypass reaction. The selectivity of Bi-PAP-A was examined using Lamda phage DNA. Bi-PAP-A could selectively detect two copies of a rare mutated allele (A190T) in the presence of as much as  $2 \times 10^9$  copies of the wild type allele in Lamda phage DNA. Bi-PAP-A was further validated using mouse genome. Spontaneous somatic mutations were directly detected in BigBlue mouse DNA at a frequency as low as  $3 \times 10^{-9}$ . Bi-PAP-A is a simple, rapid, general method capable of automation and particularly suited to detection of rare mutations.

**Comparative Gene Expression Analysis of Microdissected Brain Tissues in a Mouse Model of Idiopathic Parkinsons Disease Using a Novel RNA Amplification System.** *J. Heath<sup>1</sup>, A. Brooks<sup>2,3</sup>, E. Richfield<sup>4</sup>, M. Thiruchelvam<sup>2</sup>, D. Cory-Slechta<sup>2</sup>, M. Wang<sup>1</sup>, P. Chen<sup>1</sup>, A. Dafforn<sup>1</sup>, G. Deng<sup>1</sup>, D. Iglehart<sup>1</sup>, S. Koritala<sup>1</sup>, S. Lato<sup>1</sup>, S. Pillarisetty<sup>1</sup>, R. Purohit<sup>1</sup>, M. Herrler<sup>1</sup>, J. Stanchfield<sup>1</sup>, N. Kurn<sup>1</sup>.* 1) NuGEN Technologies, Inc., San Carlos, CA 94070; 2) Department of Environmental Medicine, University of Rochester Medical Center, NY 14642; 3) Functional Genomics Center, University of Rochester Medical Center, Rochester, NY 14642; 4) Department of Pathology, University of Rochester Medical Center, Rochester, NY 14642.

Most biological processes involve global changes in gene expression. High throughput transcriptome/gene expression analysis using microarray technology has made it possible to study these global changes in expression patterns. However, the application of these technologies has been limited by the need for relatively large amounts of RNA. Global gene expression changes in limited tissue samples, such as those obtained by microdissection and fine needle aspiration, are outside the grasp of these powerful tools. This makes the development of reliable amplification technologies essential to progress in these fields. To meet these requirements, NuGEN has developed the Ovation<sup>TM</sup> Nanosample RNA Amplification system for gene expression analysis on spotted arrays. It uses the Ribo-SPIA process, a novel rapid, isothermal, linear global mRNA amplification method. The simple three-step method produces highly reproducible amplified aminoallyl cDNA in less than 4 hours from samples as small as 1ng total RNA. Ribo-SPIA generates microgram amounts of single stranded DNA product, homologous to first strand cDNA, while maintaining excellent representation of all transcripts over a 10,000 fold dynamic range. The technology has been used to profile individual animals in a mouse model of idiopathic Parkinsons disease (PD) by correlating transcript profiles of microdissected brain tissue with behavioral phenotypes. In this study of the molecular mechanism of PD, we specifically address the question as to whether insults sustained developmentally or cumulative damage across life can contribute to the etiology of the disease.

**Gene expression and endurance training- induced changes in insulin sensitivity: the HERITAGE Family Study.**

*M. Teran-Garcia*<sup>1</sup>, *T. Rankinen*<sup>1</sup>, *T. Rice*<sup>2</sup>, *A.S. Leon*<sup>3</sup>, *D.C. Rao*<sup>2</sup>, *J.S. Skinner*<sup>4</sup>, *J.H. Wilmore*<sup>5</sup>, *C. Bouchard*<sup>1</sup>. 1) Human Genomics, Pennington Biomedical Research Center, Baton Rouge, LA; 2) Division of Biostatistics, Washington University School of Medicine, St Louis, MO; 3) School of Kinesiology, University of Minnesota, Minneapolis, MN; 4) Department of Kinesiology, Indiana University, Bloomington, IN; 5) Department of Health and Kinesiology, Texas A&M University, College Station, TX.

The beneficial effects of regular physical activity on insulin sensitivity and glucose metabolism are well documented. However, the molecular mechanisms associated with the heterogeneity in responsiveness to exercise training (T) remain unknown. The objective of this study was to find novel candidate genes for endurance T-induced improvement in peripheral insulin action by using the microarray technology. Total RNA was isolated from vastus lateralis before and after 20 weeks of exercise from individuals participating in the HERITAGE Family Study. Insulin sensitivity (S) index was derived from a FSIVGTT using MINMOD analysis. Sixteen subjects were selected for the experiment: 8 showed no changes in S (low responders; LSR) and 8 displayed marked improvement in S (high responders; HSR) after the intervention. S increase after exercise T was ~5 times greater in HSR compared to LSR (4.60.7 vs. -0.780.2U/ml/min), whereas age, BMI, body fat, and baseline S were similar between the groups. Pooled RNA samples from the HSR and LSR individuals were analyzed for differences in gene expression before and after T using microarrays. Both pre- and post-T experiments were repeated 3 times. Overall, differences between LSR and HSR were more evident in the samples obtained after T. Almost twice as many genes showed 2-fold difference in expression levels between the HSR and LSR as compared to the pre-T samples. 18 genes were consistently up-regulated (2.0), and 8 down-regulated (0.5) both at pre- and post-T in the HSR, whereas expression level of 56 and 42 genes increased and decreased, respectively, after T in the HSR group. Differentially expressed genes include those involved in pathways of glucose transport, signaling and energy metabolism, along with structural genes.

**Flow-through porous microarrays: a novel platform for gene expression profiling.** *Y. Wu<sup>1</sup>, P. de Kievit<sup>1</sup>, L. Vahlkamp<sup>1</sup>, D. Pijnenburg<sup>1</sup>, M. Smit<sup>1</sup>, M. Dankers<sup>1</sup>, M. Stax<sup>2</sup>, T. Satoh<sup>3</sup>, P.J. Boender<sup>1</sup>, C. Ingham<sup>1</sup>, H. van Damme<sup>1</sup>, T. Raap<sup>2</sup>, A.B. Chan<sup>1</sup>, R. van Beuningen<sup>1</sup>.* 1) PamGene International BV, Burgemeester Loeffplein 70a, s-Hertogenbosch, The Netherlands; 2) Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands; 3) Genome Medical Business Division, Olympus Optical Co., Ltd., Tokyo, Japan.

The application of high-density microarrays has been extremely valuable in the identification of new classes of biomarkers that could be useful for therapeutic intervention and clinical diagnosis. However, subsequent validation and use of these biomarkers require focused microarrays that offer speed, robustness and reproducibility. Methods such as quantitative PCR analysis provide tremendous sensitivity but those technologies are not easy to multiplex. A novel technology platform, a flow-through porous microarray (porous array), has been developed for rapid and robust molecular biological testing. The new concept of this technology involves the use of a porous aluminium-oxide substrate as a solid support. This facilitates the flow-through incubation of a sample solution through its structure, which assures much faster and more efficient hybridization. To assess its utility in gene expression analysis, we performed a heat shock treatment on cultured Jurkat cells and analyzed the transcriptional regulation of 23 genes. The results obtained by porous arrays were confirmed by real-time quantitative PCR. Individually labeled transcripts were generated to determine the sensitivity of these arrays. Our results show a good correlation between porous arrays and quantitative PCR (73%). The minimal detectable amount with individually labeled transcripts is 20-80 amol in 20ul solution. This enables the detection of 1 in 300,000 copies of transcripts in approximately 10ug of amplified RNA. In addition, porous arrays show uniform spot morphology, high reproducibility between arrays (10.4% CV), a broad dynamic range (4 orders of magnitude) and fast hybridization kinetics. Based on these results we conclude that the flow-through porous microarray is a novel strategy that offers speed, robustness and reproducibility for gene expression profiling.

**Highly multiplexed locus-specific SNP genotyping using high-density oligonucleotide arrays.** *M.H. Shapero, A. Loraine, W. Liu, J. Zhang, X. Di, K.W. Jones.* Genotyping Research, Affymetrix, Santa Clara, CA.

With the completion of the human genome, SNPs are emerging as the marker of choice for genetic association studies. Additionally, the ongoing international effort to build a haplotype map will further identify SNPs which are expected to improve the ability to carry out genome scans and should provide the framework for new studies designed to identify the underlying genetic basis of complex diseases. Due to these applications, there is still a need for the development of robust, cost-effective platforms for large scale SNP genotyping. We are developing a new locus-specific target preparation method called MARA (Multiplexed Anchored Runoff Amplification). The approach uses a single primer per SNP in conjunction with adaptor-ligated, restriction enzyme digested human genomic DNA. Each primer contains a common sequence at the 5' end and locus-specific sequence at the 3' end. A primary reaction generates single strand extension products which are then used in a secondary amplification with common primers. Following labeling of the target DNA, allele discrimination is achieved by hybridization to high density DNA oligonucleotide arrays. We have carried out multiplex reactions containing 250 primers and 20 ng of adaptor-ligated genomic DNA across 9 DNA samples. The results show an average call rate of 99% with 36 discordant genotype calls, attributed to 9 primers, out of 2250 (250 calls x 9 people) total calls for an overall primer failure rate of less than 5%. The same DNA samples were assayed at 775-plex with an average call rate of 98% and less than 10% overall primer failure rate. We currently are in the process of a systematic analysis of the multiplex limit using SNPs from human chromosome 21, testing 1000-, 2000-, 3000-, and 4000-plex reactions using MARA. In addition, genotyping data is being used to reconstruct haplotypes using a diverse panel of DNA samples. Potentially MARA may improve the through-put of SNP genotyping by allowing levels of multiplexing during target generation that far exceed the capacity of traditional multiplex PCR.

**An efficient highly multiplexed SNP genotyping assay and application to the International HapMap Project.** *R. Shen, W. Chang, D. Campbell, J. Chen, D. Doucet, C. McBride, J.-B. Fan, S. Shaw, M. Chee, M. Hansen, A. Oliphant.* Scientific Operations, Illumina, San Diego, CA.

Millions of single nucleotide polymorphisms (SNPs) have been identified in the human genome. It is currently impractical to perform genetic association studies utilizing all of them. However, a goal of the International HapMap Project is to identify a minimal set of SNPs that can be used to detect common haplotype patterns in multiple populations. This will enable comprehensive genome-wide genetic association studies, potentially revolutionizing the search for the genetic basis of common diseases. The goal of the HapMap project will be accomplished by carrying out large-scale genotyping in different populations. The ability both to develop and effectively utilize the HapMap for genetic association studies is dependent upon tools for large-scale genotyping that are flexible, cost-effective and easy to use.

We have developed an efficient SNP genotyping assay that is easy to use and is capable of multiplexing at greater than 1,152 simultaneous assays in a single well. The assay consists of designing two allele-specific oligos and a single locus specific oligo per SNP. The assay oligos for 1,152 loci (3,456 oligos) are combined and then hybridized to a single sample of genomic DNA. After the genomic DNA hybridization, processes are performed that remove excess and mishybridized oligos prior to an allele-specific extension reaction. Extended products are ligated by a DNA ligase, and the reaction products are PCR amplified and hybridized to a Sentrix<sup>TM</sup> Array Matrix. This assay is being used by the international community to genotype 50% of the loci for the human HapMap project and by Illuminas Genotyping Services which has run >100M genotype calls. We have utilized this platform to develop a high resolution SNP linkage panel and a higher resolution fine mapping panel. The SNP linkage panel contains approximately 4,600 uniformly spaced loci and the fine mapping panel contains 40,000 loci uniformly spaced throughout the genome.

**Accumulation of random mitochondrial DNA deletions and phenotypic consequences in aged human skeletal muscle.** *G. Stepien, B. Chabi.* Laboratoire de Nutrition Humaine, UMR INRA 1019, Clermont-Ferrand, France.

Several works supported that mitochondrial respiratory chain deteriorates with age, mostly in tissues with high-energy requirements. Damage to mitochondrial DNA (mtDNA) by reactive oxygen species is thought mainly to contribute to this impairment. However, the overall extent of mtDNA mutation in aging is still not evaluated.

To quantify mitochondrial damage in aging, we are carrying out molecular and biochemical analysis in muscle biopsies from young and old healthy subjects. Deleted mtDNA accumulation with age was followed by both real-time quantitative PCR analysis that allows us to quantify mtDNA copy number with high sensitivity and Southern-blot hybridization coupled to quantification of the radioactive signals that led us to determine deleted to full length mtDNA ratio. We first settled both techniques by quantification of mtDNA alterations in patients with mitochondrial disorders: both quantifications were performed on patient samples: skeletal muscle with large-scale mtDNA deletions, tissues and cultured fibroblasts with mtDNA depletion, and liver with mtDNA over-replication. The results obtained by the two methods showed concordant data for most of the patients.

Our results underline no increase of specific mtDNA deleted sequences with age contrary to the accumulation of single or multiple large scale deletions observed in patients with mitochondrial pathologies. Deletion events should appear randomly in all mtDNA regions with aging and more than 60 percent of mtDNA copies should be deleted in the oldest subjects (more than 80 years old). Moreover, a significant increase in mtDNA copy number (deleted and full length) was detected in old subjects. Several respiratory chain enzymatic activities were simultaneously measured in all subjects. A progressive and significant decrease of all these activities with aging was observed. However, physical activity, nutritional and hormonal status, and genetic background should be further considered as major parameters that could modulate such enzymatic activity alteration in aging.

Program Nr: 1559 from 2003 ASHG Annual Meeting

**Automated transcript mapping detects brain-specific gene transcripts in a region on 16p13 associated with autism.** *A.L. Scardovi<sup>1</sup>, C. Ottolenghi<sup>1</sup>, F. Soli<sup>1</sup>, I. Stanghellini<sup>1</sup>, E. Caffo<sup>2</sup>, A. Forabosco<sup>1</sup>.* 1) Genetica Medica, University of Modena, Italy; 2) Neuropsichiatria Infantile, University of Modena, Italy.

Genome scan linkage analyses suggest that the short arm of chromosome 16 contains a locus associated with autism. In order to detect potential tissue-specific isoforms in this region, we developed a computational approach combining retrieval of data on human EST tissular distribution with exon identification in a 10 Mb sequence that contains the markers with multipoint maximum LOD scores suggestive of linkage. We screened for putative exons by EST mapping and ranked them according to their abundance in brain libraries. Two novel genes and two isoforms of a known gene showed a pattern suggestive of high expression in brain. Selective expression in brain was confirmed by RT-PCR and further characterization of the gene structures and expression patterns is under way. This approach may be highly effective in pointing to expression-based candidate genes in large genomic regions associated with complex traits.

Program Nr: 1560 from 2003 ASHG Annual Meeting

**DNA preservation using genomic libraries constructed with standard plasmid vectors.** *R. Dana.* Committee for World Health, Foothill Ranch, CA.

Preservation of large fragments of genomic DNA (100-200 kB) with bacterial artificial chromosome (BAC) vectors may also be performed with many of the standard plasmid vectors used for gene cloning. The hypothesis that DNA rearrangements are minimized when only one copy of the construct is present per bacterial cell does not appear to be true. Thus, large DNA genomic libraries may be constructed and provide a stable system for long term DNA preservation and renewal with many of the standard plasmids. These results suggest that the wide variety of cloning vectors which are now available should be evaluated with large DNA methodologies, thereby facilitating genomic research. These findings can save time and lower the cost of DNA preservation research.

**Shuffling of genes within low copy repeats on 22q11 (LCR22) by Alu-mediated recombination events during evolution.** *B.E. Morrow<sup>1</sup>, A. Pavlicek<sup>2</sup>, M. Babcock<sup>1</sup>, C.D. Kashork<sup>3</sup>, L. Shaffer<sup>3</sup>, J. Jurka<sup>2</sup>.* 1) Dept Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) Genetic Information Research Institute, Mountain View, CA; 3) Health Research and Education Center, Washington State University, Spokane.

Low copy repeats or segmental duplications are highly dynamic regions in the genome. Low copy repeats on chromosome 22q11.2 (LCR22) mediate chromosome rearrangements associated with velo-cardio-facial syndrome/DiGeorge syndrome, der(22) syndrome and cat-eye syndrome. The LCR22s are a complex mosaic of genes and pseudogenes, formed by duplication and transposition processes. The ability to trace the substrates and products of recombination events provide a unique opportunity to identify the mechanisms responsible for shaping LCR22s. We examined the genomic sequence of known LCR22 genes and their duplicated derivatives. We found Alu (SINE) elements at the breakpoints in the substrates and at the junctions in the products of recombination for USP18, GGT and GGTLA, consistent with Alu-mediated unequal crossing-over events. In addition, we were able to trace an Alu-mediated rearrangement event between IGSF3 on 1p13.1 and GGT on 22q11.2. Most breakpoints examined occurred in the 5' or 3' ends of the Alu elements. A possible stimulus for these rearrangements may be the high sequence similarities between different Alu elements, combined with the potentially recombinogenic role of retrotransposon target site duplications flanking the Alu element, containing potentially kinkable DNA sites. Such sites may represent focal points for recombination. Thus, genome shuffling by Alu-mediated rearrangements has contributed to genome architecture during primate evolution. The availability of individuals with chromosome rearrangement disorders provides an additional opportunity to understand recombination mechanisms in a single meiosis. It is possible that some of the same mechanisms are responsible for both events.

**Genomic architecture involved in *PLP1* duplication causing Pelizaeus-Merzbacher disease.** *J.A. Lee*<sup>1</sup>, *M. Dean*<sup>3</sup>, *B. Gold*<sup>3</sup>, *J.R. Lupski*<sup>1,2</sup>, *K. Inoue*<sup>1</sup>. 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pediatrics, Baylor College of Medicine, Houston, TX; 3) Human Genetics Section, Laboratory of Genomic Diversity, NCI-Frederick, Frederick, MD.

Genomic architecture, a higher order structural feature of the human genome, can provide molecular substrates for recurrent submicroscopic chromosomal rearrangements, including deletion and duplication. Such rearrangements lead to gene dosage alterations within the rearranged genomic segment that may result in genomic disorders. Many disease-causing genomic rearrangements are mediated by homologous recombination between flanking low-copy repeats (LCRs), yielding common recombinant genomic segments. Pelizaeus-Merzbacher disease (PMD) is a genomic disorder that most commonly (60-70%) arises from genomic duplications of the dosage-sensitive proteolipid protein gene (*PLP1*). Interestingly, the breakpoints of the *PLP1* duplication are not common, yielding duplicated genomic segments of varying lengths. This suggests that the molecular mechanism underlying *PLP1* duplication events is likely distinct from the common mechanism. To determine whether genomic architecture also facilitates *PLP1* duplication events, we performed large-scale genome sequence analyses and identified several LCRs within the *PLP1* region. To further examine whether these LCRs may stimulate the genomic rearrangements leading to *PLP1* duplications, we employed pulsed-field gel electrophoresis (PFGE). PFGE analyses using probes adjacent to these LCRs detected recombination-specific junction fragments in 6 of 9 families with *PLP1* duplications. These data suggest that these LCRs may be involved in mediating the duplication events. Our study provides evidence that genomic architectural features may result in susceptibility to the genomic duplications responsible for the majority of PMD cases.

**Human chromosome 7: DNA sequence, biology, and medical genetics.** *S.W. Scherer*<sup>1,2</sup>, *J. Cheung*<sup>1</sup>, *L.R. Osborne*<sup>2</sup>, *J.R. MacDonald*<sup>1</sup>, *K. Nakabayashi*<sup>1</sup>, *J. Herbrick*<sup>1</sup>, *J. Skaug*<sup>1</sup>, *R. Khaja*<sup>1</sup>, *J. Zhang*<sup>1</sup>, *M. Li*<sup>1</sup>, *M. Haddad*<sup>1</sup>, *A.R. Carson*<sup>1</sup>, *L. Parker-Katirae*<sup>1</sup>, *G.E. Duggin*<sup>1</sup>, *J. Vincent*<sup>1</sup>, *J.M. Rommens*<sup>1,2</sup>, *J.C. Venter*<sup>3</sup>, *R.J. Mural*<sup>3</sup>, *M.D. Adams*<sup>3</sup>, *L.-C. Tsui*<sup>1,2</sup>. 1) The Centre for Applied Genomics, Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Molecular and Medical Genetics, University of Toronto, ON, Canada; 3) Celera Genomics, Rockville, MD, USA.

We have generated an assembly of the complete DNA sequence of human chromosome 7 and an initial annotation of the biological features relevant to development, variation, and disease (*Science*, 2003). In order to establish the most comprehensive sequence database, we combined unpublished Celera scaffolds and our new sequence (comprising 85% of the assembly) with data from public repositories (15%). The compilation consisted of 157,953,789 bp encompassing all DNA markers and known genes. In our dataset there were 1,022,294 bp of sequence not present in the last NCBI build, 204,066 bp at 8 sites inverted between assemblies, and 508,332 bp at 132 sites with variable sequence. The DNA sequence framework served as a reference for annotation of all relevant molecular and biomedical information. We identified 1917 genes and gene segments; 100 of these were not in other databases, and for another 200 our description was more complete. In addition, an international group of 70 scientists from 10 countries (for complete list of authors see *Science*, 2003) contributed new experimental data and this work continues. Over 1400 clinical records of individuals having defined phenotypes and chromosome 7 rearrangements have been catalogued. For 500 of these, the breakpoints were precisely mapped along the DNA sequence providing a rich resource for medical genetic discovery (we provide examples of how this has been used to study autism). To facilitate further community-based structural and functional annotation of human chromosome 7 we have implemented the Generic Model Organism Database to display all of this chromosome 7 data (<http://www.chr7.org/>), which is continually updated and curated.

**Genomic inversion polymorphism in parents of patients with Williams-Beuren syndrome.** *K. Wakui<sup>1</sup>, K. Inoue<sup>1</sup>, C. Kashork<sup>1</sup>, L.G. Shaffer<sup>1,4,5</sup>, J.R. Lupski<sup>1,2,3</sup>*. 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Children Hospital, Houston, TX; 4) Health Research and Education Center, Washington State University, Spokane, WA; 5) Sacred Heart Medical Center, Spokane, WA.

Williams-Beuren syndrome (WBS; MIM #194050) is a contiguous gene syndrome caused by a deletion of multiple genes within chromosome 7q11.23. The common ~1.5 Mb deletion likely results from non-allelic homologous recombination between directly oriented DNA blocks of low-copy repeats, LCR7s. Recently, inversion of the same segment has been described as a polymorphic variant in 4 of 12 (33%) parents of WBS patients with common deletion, as well as in 1 of 8 atypical WBS patients without deletion, and 3 atypical WBS patients with balanced chromosomal rearrangement (Osborne et al. 2001). We studied 15 WBS families by PFGE analysis, and identified the presence of 7 PFGE junction fragments in parents (47%). Our data confirm the previous findings of high rate of genomic inversion polymorphism in parents of patients with WBS, and support the hypothesis that the presence of a heterozygous inversion polymorphism may increase susceptibility to non-allelic homologous recombination that results in WBS deletion.

**The search for genetic modifiers of asthma through expression profiling of peripheral blood lymphocytes. S.**

*Hilmer<sup>1</sup>, N.N. Hansel<sup>2</sup>, S.N. Georgas<sup>2</sup>, L.M. Cope<sup>2</sup>, J. Guo<sup>2</sup>, R. Irizarry<sup>2</sup>, E.P. Hoffman<sup>1</sup>, G.B. Diette<sup>2</sup>.* 1) Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 2) Department of Pulmonary and Critical Care Medicine, The Johns Hopkins Hospital.

Asthma is a common chronic inflammatory disease of the lungs, which affects over 10 million adults in United States alone. This disease has considerable public health consequences due to the enormous variability in disease progression. As a result of this variability, those patients with more severe disease have a disproportionately high morbidity, mortality risk, and impact on the health care system. Additionally, severe and poorly controlled asthma may lead to inappropriate tissue remodeling which results in irreversible disease and prolonged disability. Although a few candidate genes have been suggested, research focused on the possible genetic predisposition for severe asthma is scarce. Furthermore, little is known about the molecular mechanisms underlying disease progression and degrees of severity. CD4+ Th2 lymphocytes may play an important role in airway inflammation and hyper-responsiveness. However, the relationship between lymphocytes and their effector function in asthma progression has not been fully explored. Here we aim to develop a better understanding of lymphocyte activity through expression profiling using the Affymetrix Human Genome 133 GeneChip series. The up-regulation of genes involved in the Ras-MAPK pathway (namely, RAB14, RAB2, KRAS2 and CAPRI) in severe asthmatics, suggests the importance of T cell proliferation in asthma severity. Severe asthmatic subjects also had increased expression in genes involved in T cell activation (CD6, CD3E), cytoskeletal changes (ARPC4, PLEC1, RAC2, SEPT1) and cell adhesion (NK4, B4ALT1) supporting the concept of lymphocyte recruitment to tissues or areas of inflammation. Comparing and contrasting transcriptional programs of severe and mild asthmatics may contribute to a better understanding of asthma severity and progression as well as lead to the identification of markers for those who have an increased risk of severe asthma.

**Transcriptional profiling of adult mouse muscle and bone marrow SP cells.** *K.N. Liadaki, D. Sanoudou, J. Schienda, A. Kho, A. Beggs, L.M. Kunkel.* Harvard Medical School, Children's Hospital, Boston, MA.

Stem cells have the capacity to self-renew and the ability to generate differentiated cells. The identification of genes that govern the specific properties of stem cells is important for basic cell biology and stem cell-based therapy. Stem cell sources vary depending on the tissue and the isolation procedure. A technique based on the exclusion of the DNA binding dye Hoechst 33342 has been used to identify and purify, using a fluorescence activated cell sorter (FACS) a sub-population of cells, termed side population (SP) cells, from the main population (MP) cells. SP cells have been isolated from adult bone marrow (BM) and skeletal muscle and are enriched in stem cell activity, based on the expression of stem cell markers, and on transplantation experiments. We used the Affymetrix U74Av2 microarrays to characterize the transcriptional profiles of over 12000 probe sets in SP cells isolated from murine adult skeletal muscle and BM. We have found significant changes in the gene expression involved in multiple cellular pathways including signal transduction pathways (11%) (specifically receptor molecules -8%), regulation of cell cycle and development (7%) and transcription/translation mechanisms (11%). A large number of genes of unknown molecular function (25%) were significantly up-regulated in SP, compared to MP cells, and are currently under investigation. Although many differences were evident between muscle and BM SP cells, their similarities may enable the identification of common pathways of SP function in different tissues, as well as the identification of tissue-specific SP markers, which could facilitate the isolation of SP cells and improve their engraftment into recipient tissues.

**The Effect of Vitamin E on Gene Expression in Patients With Age-Related Macular Degeneration (AMD).** *J.D. Flippin<sup>1</sup>, N. Strunnikova<sup>2</sup>, S.C. Hilmer<sup>1</sup>, E.P. Hoffman<sup>1</sup>, K.G. Casky<sup>2</sup>*. 1) Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 2) National Eye Institute, National Institutes of Health, Bethesda, MD.

One in three people 65 and over has a form of eye disease. Of these diseases Age-Related Macular Degeneration (AMD) is the leading cause of blindness. With the baby boomer generation reaching this age category, the need to better understand the disease is crucial. While there is significant use of antioxidants in clinical trials, it is important to understand the pathogenesis of the disease. The eye is susceptible to oxidative stress due to a high exposure to sunlight, content of polyunsaturated fatty acids, and consumption of oxygen. In AMD patients there seems to be abnormal cell response to the injury caused by this oxidative stress. It has been reported that similar abnormal cell response to injury is occurring in other tissues like skin. Although there is no pathological phenotype, there is a change of histology of the skin of AMD patients. Ironically, like the eye, the skin has a high exposure to sunlight making it susceptible to oxidative stresses similar to those affecting the eye. Our study uses dermal fibroblast cells from patients with either nonexudative form of AMD; Drusen or Geographic Atrophy. Cultured fibroblasts isolated from patients were expression profiled both pre- and post- injury induced with menadione both with and without pre-treatment with vitamin E. Expression changes were evaluated for the 22,284 genes and ESTs represented on the Affymetrix HG\_U133A GeneChip. Results indicate differential expression of genes with known antioxidant function, including antioxidant protein 1 (ATX 1) and genes with important roles in lipoprotein inhibition such as tissue factor pathway inhibitor (TFPI). The results of our study have important implications for understanding the pathogenesis of AMD and the use of antioxidants in treatment regimes.

**Haplotype-specific BAC libraries for identifying amyotrophic lateral sclerosis (ALS) loci through integrative genomics.** *Y. Yoshinaga, M. Nefedov, K. Osoegawa, P.J. de Jong.* BACPAC Resources, Children's Hospital Oakland Research Institute, Oakland, CA.

Complex traits are caused by variation at multiple interacting loci. The underlying genetic defects are thus difficult to identify and require genetic analysis from small pedigrees to ensure that all cases are inheriting the trait in a Mendelian way. We are collaborating with a consortium of research groups with access to distinct pedigrees of familial ALS patients with dominant disease-predisposing alleles. The small pedigrees permit genetic localization to chromosomal regions in the range of 5-10 cM. To identify the predisposing alleles, multiple sources of information will be combined to indicate likely candidate FALS genes. One source of information is the complete haplotype-specific sequence for the affected chromosomal interval. This can conceivably be accomplished through a long-range PCR strategy or a molecular cloning approach. Molecular cloning has the advantage that single DNA molecules are isolated and cloned, thus establishing a single haplotype intermediate for sequencing. The use of human-mouse hybrid cell lines facilitates this approach by providing an option for the selective cloning BACs from a single haplotype, thus reducing the need for high-resolution haplotype mapping to distinguish clones derived from the allelic autosomes. Using the completed human genome sequence, synthetic probes can be designed at regular intervals and pooled for library screening. This permits the rapid isolation of a BAC contig and the identification of a minimal set of BAC clones defining the region, to be used for the shotgun sequencing of the affected regions. We have prepared two BAC libraries derived from FALS patients with predisposing alleles on human chromosome 16 and 18, and are progressing toward the mapping of the affected chromosomal regions. In addition to providing an intermediate for sequencing the affected haplotypes, the BACs also provide a source of candidate genes for functional gene assay, for instance by integrating EGFP reporter constructs for cell based or transgenic animal approaches. Our research is supported in part by a grant from the ALS Association.

**HLA typing for determination of type 1 diabetes associated DQA1, DQB1 and DRB1 alleles.** *M. Sjoroos, J. Seppala, H. Grey.* Life & Analytical Sciences, PerkinElmer, Turku, Finland.

The most important gene loci defining risk for type 1 diabetes are located within HLA region. The HLA-DQ molecules are of primary importance, but HLA-DR products can modify the risk conferred by HLA-DQ. Thus, HLA-DQA1, -DQB1 and DRB1 assays for the detection of type 1 diabetes associated alleles (alleles associated with risk or protection) were developed for pathogenesis and prevention studies. Currently, these assays are used as large-scale screening tests for the risk estimation of type 1 diabetes. The assays are based on the combined use of the polymerase chain reaction (PCR) amplification of the relevant exon 2 polymorphic regions of the three genes and solution hybridization with lanthanide-labeled allele-specific oligonucleotides using time-resolved fluorometry for detection. The biotinylated PCR products are attached onto streptavidin-coated microtitre wells for hybridization. After a denaturation step, the hybridization reactions are carried out with europium, samarium or terbium-labeled probes. For the interpretation of the results, a MultiCalc can be used. The assays support a direct use of dried blood spots as template material in PCR without any sample pre-treatment steps. Alternatively, isolated genomic DNA can be used as well. To confirm the specificity of the three assays, selected samples were genotyped using sequencing as a reference method. Method comparison with sequencing showed a 100 % concordance in recognizing all the probe specific alleles present.

**Megabase Linkage Disequilibrium in *Canis familiaris*.** *N.B. Sutter*<sup>1</sup>, *H.G. Parker*<sup>1</sup>, *M.A. Eberle*<sup>1</sup>, *E.F. Kirkness*<sup>2</sup>, *E.A. Ostrander*<sup>1</sup>, *L. Kruglyak*<sup>1</sup>. 1) Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) The Institute for Genomic Research, Rockville, MD.

The 156 breeds of registered dogs in the U.S. offer an unparalleled opportunity to map genes important in disease susceptibility, morphology, and behavior. Linkage disequilibrium (LD) is of current interest for its application in whole genome association mapping, since the extent of LD determines the feasibility of such mapping. We have measured LD at two loci, neither of which is under obvious selective pressure, in the Golden Retriever, Pekingese, and Akita breeds in order to evaluate the utility of association mapping in the canine. The Golden Retriever was created only 150 years ago from the mixing of several distinct breeds reported to include the Tweed Water Spaniel, Yellow Retriever, Bloodhound, and Irish Setter. The breed as a whole has not experienced any major population bottlenecks. By comparison, the Pekingese is an ancient breed, and its population in the U.S. is purportedly derived from a small number of founders. The Akita has been geographically isolated until this century, when small numbers founded the U.S. population. To create a marker set suitable for measuring LD, we carried out SNP discovery by resequencing major portions of two sequenced BACs. Both BACs map to canine chromosome 14. Five unrelated dogs from each breed were used for SNP discovery; 252 SNPs were found in 130 Kb of sequence. Common SNPs in each breed were then genotyped by resequencing in an additional 15 unrelated dogs. Using the  $D$  and  $r^2$  statistics, we find that in both breeds LD extends with little or no diminution for over 2 Mb at one locus, but does not extend between the two loci (50 Mb). LD in canines appears more extensive than in humans; we are working to characterize its precise extent.

Program Nr: 1571 from 2003 ASHG Annual Meeting

**Knowledge-Driven Selection of SNP Markers for Genetic Association Studies of Complex Diseases.** *H. Avi-Itzhak, F.M. De La Vega.* Applied Biosystems, Foster City, CA.

Applied Biosystems developed a first generation whole-genome, gene-centric reference SNP map for use in candidate-gene, candidate region, and eventually whole-genome disease association studies. The selected candidate SNPs were validated by individually genotyping 180 DNA samples from African-American, Caucasian, Chinese, and Japanese individuals. The current set of validated markers comprises over 120,000 SNPs of high heterozygosity in at least one population available as ready to use 5 nuclease assays with TaqMan MGB probes. The individual genotypes generated thus far in the validation have enabled us to survey the profile of linkage disequilibrium (LD) and haplotype diversity across most gene regions of the human genome. This data offers a foundation for an empirically driven, rational design of association studies according to the specific population and region of the genome of interest. We developed a visualization tool to represent the location of SNPs, genes, and haplotype blocks across both physical and LD maps. These LD maps use a metric unit system developed by Maniatis et al., (PNAS 99: 2228-33, 2002) that reflects the variation in the strength of LD across genomic regions. In the design of association studies, it is often useful to examine the impact of making different choices for variables such as sample size, assumptions on the disease relative risk, mode of inheritance, as well as mutation allele frequency, taking into account the empirically determined patterns of LD at each loci of interest. The summary of power calculations under the assumptions of the common variant/common disease hypothesis is also represented in the visualization. Finally, we developed strategies to show SNP type, population-specific minimum-subsets of SNPs (tagging SNPs), and for identifying regions that will benefit from supplementary SNPs. The visualization tool presented here is intended to guide the cost-effective selection of validated SNP assays for the design of genetic association studies exploiting the a priori knowledge of the LD profile obtained on a reference set of population samples.

**Whole-genome amplification template combined with highly multiplexed SNP typing enables large-scale association studies from archived DNA samples.** *R. Pask, H. Rance, N. Walker, A. Lam, L. Smink, D. Smyth, B.J. Barratt, J.A. Todd.* JDRF/WT Diabetes and Inflammation Laboratory, CIMR, University of Cambridge, Cambridge, UK.

Sustainable DNA resources and reliable genotyping methods are essential to any large genetic association study. In the genetic dissection of common disease it is now recognised that thousands of samples and tens of thousands of markers, mostly single nucleotide polymorphisms (SNPs), will have to be analysed. To help alleviate the burden on DNA stocks and genotyping budgets this imposes, we have investigated Molecular Staging's Multiple Displacement Amplification (MDA) template in combination with Illumina's highly multiplexed BeadArray genotyping technology.

The MDA method for whole-genome amplification provides a uniform representation of all loci tested so far across the genome. After several successful small scale studies, we carried out a 100-SNP evaluation using ABI's Taqman Assay on 88 individuals, directly comparing genomic DNA (gDNA) with MDA, obtaining a concordance rate of >99.7%. The failure rate was acceptable, <2% higher for MDA than for gDNA. We then investigated the compatibility of MDA with Illumina's BeadArray method. We compared MDA to gDNA template by genotyping 86 samples at 345 SNPs. For a subset of 15 SNPs against >3000 samples we compared Illumina-generated genotypes with data generated by other technologies. In the 86-sample MDA versus gDNA comparison, the concordance rate in Illumina data was 98.8%. In the 15-SNP subset there was a concordance rate of 99.6% between Illumina and other technologies. The data Illumina presented was almost complete (>99.9%), however, samples that performed badly, 10.5% for MDA compared to 5.8% for gDNA, were excluded.

Our evaluation demonstrates that Molecular Staging's MDA template is compatible with Illumina's BeadArray technology. In combination, these technologies solve the problems of stock DNA limitations and provide a part-solution to the cost problems of ultra high throughput genotyping.

**Physical and transcript map of the autosomal dominant colobomatous microphthalmia locus on chromosome 15q12-q15 and refinement to a 3.4 Mb region.** *L. Michon*<sup>1</sup>, *L. Morle*<sup>1</sup>, *M. Bozon*<sup>1</sup>, *L. Duret*<sup>2</sup>, *J.-C. Zech*<sup>3</sup>, *J. Godet*<sup>1</sup>, *H. Plauchu*<sup>4</sup>, *P. Edery*<sup>5</sup>. 1) Molecular and Cellular Biology, CNRS, LYON, FRANCE; 2) Biometry and Evolutive Biology, CNRS, LYON, FRANCE; 3) Ophthalmology Service, Hospices Civils de Lyon, LYON, FRANCE; 4) Genetics Service, Hospices Civils de Lyon, LYON, FRANCE; 5) Genetics Unit, Hospices Civils de Lyon, LYON, FRANCE.

Congenital microphthalmia is a developmental disorder characterized by shortened axial length of the eye. We have previously mapped the gene responsible for autosomal dominant colobomatous microphthalmia in a 5-generation family to chromosome 15q12-q15. Here, we set up a physical and transcript map of the 13.8 cM critical region, bounded by loci D15S1002 and D15S1040. This region includes 5 contigs containing 17 novel polymorphic markers and at least 84 genes and 38 retinal ESTs. Physical mapping and genetic linkage analysis in this microphthalmia family allowed the refinement of the disease locus to two intervals in close vicinity, namely a centromeric interval, bounded by microsatellite DNA markers m3 and m17, and a telomeric interval, D15S165-m25, encompassing respectively 0.7 and 2.7 Mb. Thereafter, we excluded three candidate gene, *CKTSF1B1*, *KLF13* and *CX36*. In addition, as a phenomenon of anticipation was suggested by phenotypic and pedigree data, we analyzed 3 trinucleotide repeats located within the telomeric candidate interval and found no abnormal expansion in affected individuals compared to controls. We are now carefully checking new information on chromosome 15 DNA sequence annotation in order to identify the disease-causing gene.

**Efficient Construction of User Specified Genetic Maps Based on Publicly Available Physical and Genetic Map**

**Data.** *A. Pluzhnikov*<sup>1</sup>, *W. Wen*<sup>1</sup>, *S. Luban*<sup>2</sup>, *Y. Chen*<sup>1</sup>, *N.J. Cox*<sup>1</sup>. 1) Department of Human Genetics, The University of Chicago, Chicago, IL; 2) Department of Computer Sciences, Purdue University, W. Lafayette, IN.

Creating accurate fine scale genetic maps is a crucial step in human genetic analysis. We have developed a fast and efficient set of tools that enables a user to generate a genetic map for a specified set of markers. The tools consist of programs (Perl scripts) and data sets containing the current version of human genome assembly STS and RefSeq gene maps (downloaded from [genome.cse.ucsc.edu](http://genome.cse.ucsc.edu)) and two commonly used genetic maps (Marshfield and deCode) in a flat file format, as well as the files containing Marshfield's cryptic duplicate marker names and alias names for all markers in the STS map. As a preliminary step, comprehensive joint physical/genetic maps were constructed for both Marshfield and deCode markers (available at <http://hg-wen.uchicago.edu/maps>) comprising 97% of Marshfield and over 99% of deCode markers. A marker name supplied by the user is matched against these maps to obtain the physical location of the marker and its coordinate in one or both of the genetic maps, if available. If a match is not found, we proceed by trying to match any of the available aliases or cryptic duplicates associated with this marker. Those markers that are not found can still be located in the physical map, and their genetic map location be interpolated using the genetic and physical map coordinates of two closest flanking markers. It should be noted that the procedure is purely deterministic. The resulting map can be used on its own, or as a starting point to improve convergence and accuracy of likelihood-based algorithms for map construction, e.g. CRIMAP.

All data are stored and accessed locally rather than online thus greatly reducing execution time and allowing for more flexibility in exchange for a tolerable increase in memory and disk space usage (< 30 Mb in total).

**Sizing of long DNA fragments by capillary gel electrophoresis: Practical issues for genetic analysis.** *M. Dobbs, B. Archer.* Biomedical Research Division, Beckman Coulter, Inc., Fullerton, CA.

Most STRs can be genotyped by analyzing amplicons of less than 500 nucleotides in length. There are a number of applications, however, where reproducible sizing of longer fragments is required. Three of the major sources of difficulty in the sizing of longer DNA fragments are:

- Insufficient electrophoretic resolution

- Migration effects due to sequence differences between unknown samples and size standards

- Size standard curve-fitting limitations

A group of highly polymorphic STRs was initially typed on a set of random human genomic DNAs using closely flanking primers, and then with more distantly flanking primers. A comparison of allele fragment sizes between closely flanking and distantly flanking primers demonstrated reproducible and consistent size estimates to nearly 1000 nucleotides when analyzed by capillary gel electrophoresis. Several different curve fitting models and strategies were examined for effectiveness in precise DNA fragment sizing and binning on Beckman Coulter's genetic analysis system. In general, a subset of size standards that most closely flanked the size range of interest enabled the best curve fitting. The ability to reproducibly estimate the sizes of fragments up to 1000 nucleotides in length has implications for sizing longer STRs as well as for multiplex primer design and data analysis. Several practical considerations in performing the sizing will be discussed.

Program Nr: 1576 from 2003 ASHG Annual Meeting

**A Comparison of Multiple Microarray Platforms for Gene Expression.** *S. Nelson, B. Merriman.* Rm 5506b Gonda, UCLA Medical Ctr, Los Angeles, CA.

There are currently a variety of microarray platforms available for doing whole genome gene expression measurement. A new Microarray Consortium sponsored by the NINDS and NIMH has been chartered to provide expedient access to such technologies for sponsored researchers. As a first phase of this effort, we Consortium members have undertaken a large scale comparison of the currently available technologies, in order to characterize the levels of repeatability, accuracy, sensitivity and agreement between the diverse platforms. In this report, we summarize our findings. The study is based upon assaying aliquats from the same four total RNA samples (samples derived from bulk Human Liver, Kidney, and Spleen, plus the Stratagene Human Reference pool) on diverse platforms, each sample done in triplicate, with the assays carried out at centers/facilities well-versed in the respective techniques. The platforms include whole genome pre-fabricated oligo arrays from Affymetrix, Agilent and Amersham, plus custom spotted arrays from whole genome oligo sets from Operon, ClonTech and Sigma, plus 33,000 clone custom spotted cDNA arrays. In addition to testing for repeatability and consistency, independent validation of accuracy and sensitivity is provided by assaying the same samples via large scale quantitative RT-PCR (over 1000 genes assayed by the Standard Expression Measurement Center), targeted RT-PCR on the 100 genes most discordant between microarray platforms, and SAGE analysis (a form of direct molecular transcript counting). We summarize the many dimension in which these approaches agree and disagree, although overall there is good repeatability and concordance between the major commercially fabricated microarray platforms, as well as spotted cDNA arrays.

**FVBS/Ant, a sighted variant of the FVB/N strain suitable for behavioral analysis.** *R.F. Kooy<sup>1</sup>, V. Errijgers<sup>1</sup>, I. Gantois<sup>1</sup>, G. Nagels<sup>2</sup>, A.W. Grossman<sup>3</sup>, P.P. De Deyn<sup>2</sup>, R. D'Hooge<sup>2</sup>.* 1) Dept Medical Genetics, Univ Antwerp, Antwerp, Belgium; 2) Dept Neurochemistry and Behavior, Univ Antwerp, Belgium; 3) Beckman Institute, University of Illinois, Urbana-Champaign, IL, USA.

Mice of the FVB/N strain are severely visual impaired as a result of genetic defects in the tyrosine kinase gene and the cGMP phosphodiesterase gene, resulting in albinism (*c/c*) and retinal degeneration (*rd/rd*), respectively. Nevertheless, FVB/N mice are commonly used for the generation of transgenic animals because of their large, strong pronuclei and high breeding performance. However due to visual impairment of the FVB/N animals, the resulting transgenic animals cannot be used in tests that depend on vision, including tests of cognitive behavior. Therefore we have bred a sighted version of FVB/N by intercrossing the FVB/N strain with 129P2/OlaHsd followed by repeated backcrossing with FVB/N mice while selecting against albinism and homozygosity of the retinal degeneration mutation. After 11 generations of backcrossing, sighted animals were intercrossed to generate the congenic FVBS/Ant strain, that is pigmented (*c-ch/c-ch*) and devoid of the genetic predisposition to retinal degeneration. The visual abilities of the FVBS/Ant mice were demonstrated by eye histology, a clear visual evoked potential in response to light stimuli and by increased performance in the Morris water maze test.

**Microarray-based molecular diagnosis of calpainopathy.** *M. Bakay, Y. Chen, E.P. Hoffman.* Center for Genetic Medicine, Children's National Medical Center, Washington, DC.

Most monogenic defects have straightforward molecular diagnostic tests. In the muscular dystrophies, biochemical testing of biopsies has remained one of the front line methods of providing a molecular diagnosis, however one of the more common muscular dystrophies (LGMD2A; calpainopathy) has proven difficult to diagnose with either DNA or protein-based tests. Indeed, there are no clinical or research services for providing a diagnosis for this most common form of limb-girdle muscular dystrophy.

To develop a novel molecular diagnostic approach, we hypothesized that an RNA-based expression signature might prove to be a diagnostic method of choice for the calpainopathies. Towards this end, we have expression profiled 54 muscle biopsies with Affymetrix U133A chips for a series of muscular dystrophies (BMD, DMD, FKRP, Dysferlinopathy, and Calpainopathy with 5, 9, 7, 9, and 12 samples in the group respectively) and normal control group (12 samples) to derive a specific expression signature potentially diagnostic of calpainopathies. Both MAS5.0 and dChip probe set analysis software were used. We also compared data both before and after streptavidin-phycoerythrin amplification of microarrays.

The calpainopathy profiles showed significant signatures, although they varied dependent on analysis method (12 diagnostic genes by dChip and 61 by MAS5.0). These calpainopathy-specific genes are likely to shed light on the pathophysiology of this disorder, while also permitting test of RNA-signature-based molecular diagnostics. The sensitivity and specificity of the microarray analysis methods will be presented, as well as the effectiveness of the signature of identifying LGMD patients harboring calpain III mutations.

**Muscle specific transcriptional changes in nemaline myopathy mice.** *D. Sanoudou<sup>1</sup>, M.A. Corbett<sup>2</sup>, J.N. Haslett<sup>1</sup>, M. Han<sup>1</sup>, L.M. Kunkel<sup>1</sup>, E.C. Hardeman<sup>2</sup>, A.H. Beggs<sup>1</sup>.* 1) Dept Genetics, Enders 5, Harvard Med Sch/Children's Hosp, Boston, MA; 2) Children's Medical Institute, Wentworthville, Australia.

Nemaline myopathy (NM) encompasses a clinically and genetically heterogeneous group of disorders characterized by nemaline rods and skeletal muscle weakness. Mutations in 5 sarcomeric thin filament genes (including alpha-tropomyosin) have been identified. We previously reported significant transcriptional changes in glycolytic pathway, muscle satellite cell and connective tissue related genes, in NM patients skeletal muscle.

Using Affymetrix U74Av2 GeneChips we analyzed the expression patterns of ~12,000 probe sets in 5 different skeletal muscles of transgenic alpha-tropomyosin-slow (Met9Arg) and wild type (wt) mice. For each muscle 8 independent RNA pools (from 3-5 mice each) were generated (4 wt and 4 transgenic). Significance analysis of microarrays (SAM) was used to identify genes with significant expression differences between wt and transgenic muscles.

Dramatic disease related differences were evident between the 5 muscle groups studied in transgenic compared to wt mice. At a false discovery rate <12%, diaphragm had 1,524 significantly changed probe sets while, at the other extreme, extensor digitorum longus had only 4. In diaphragm 99.6% of significantly changed probe sets were underexpressed, yet in all other muscles 100% of altered transcripts were overexpressed. The most similar muscles were tibialis anterior and plantaris, sharing 19 of their altered probe sets. Only two genes were consistently overexpressed across 4/5 muscles: *Ankrd2*, a putative sarcomeric gene, and *Csrp3*, thought to be myogenesis-related.

Determining muscle specific transcriptional changes will point to similarities characteristic of the primary steps in disease pathogenesis. Identifying mouse muscle groups best reflecting the pathogenic transcriptional changes seen in NM patients may serve as a reliable experimental system for further study of disease development and potential evaluation of therapeutic targets.

Program Nr: 1580 from 2003 ASHG Annual Meeting

**Large scale variation scanning using Mismatch Repair Detection (MRD).** *M. Faham, H. Fakhrai Rad, J. Zheng, K. Wong, M. Moorehead, A. Boudreau, J. Eberle, C. Bruckner, H. Jones, M. Jain, T. Willis.* ParAllele BioScience, SoSan Francisco, CA.

In order to directly identify variations involved in complex disorders, we have developed a technology which can be used to search comprehensively for variations in large numbers of genes. This technology which we call Mismatch Repair Detection (MRD) is a multiplexed variation scanning technology capitalizing on the bacterial mismatch repair system. A bacterial strain called the Mutation Sorter Strain has been engineered to be able to sort amplicons from thousands of genic regions (typically exons) into those that are identical to a reference sequence and those that contain variations. The fragment content of these 2 pools can then be identified using a standard tag chip readout. We have demonstrated this technology by investigating a linkage peak in a case/control study for a complex disease. The study examined all coding sequence for 160 candidate genes in 425 patients and controls. For each patient we have tested ~1,000 amplicons making the total number of amplicons analyzed close to a million and the total DNA sequence analyzed ~300 Mb. Using this technology we achieved a throughput of 30 Mb of sequence analyzed per day using 4 FTEs. Results of this scan are presented.

**De Novo Human Germline Mutations: Enhanced Mosaicism for G:C to A:T nonCpG transitions and the first observation of a single nucleotide bi-mutation are consistent with events in early embryogenesis.** X. Li, A. Halangoda, A. Karlea, W. Scaringe, K. Hill, S. Sommer. Molecular Genetics, City of Hope Medical Center, Duarte, CA.

Mutation origins are difficult to delineate. In the largest collection to our knowledge, analysis of 59 mutation origins in the Factor IX gene (F9) showed that Bayesian-based sex ratios of F9 germline mutation vary significantly with mutation type (Ketterling et al. 1999 Hum Genet 105:629). We now double the sample size of F9 origins and triple the sample size when Factor VIII (F8) and Duchenne muscular dystrophy (DMD) mutation origins are added. Among >770 independent mutations in F9, 61 male and 67 female origins and 10 mosaics were determined by direct examination of genomic DNA from the origin individuals blood leukocytes. Dramatic differences occur in the male to female sex ratio of G>A relative to A>G transitions (1.35 vs. 8.9). Six of the mosaics were G>A non-CpG mutations, consistent with the recent observation that nonCpG methylation occurs in early embryogenesis. Interestingly, the first case of a nucleotide bi-mutation was identified. Two different mutations at the same base position were identified in a hemophilia A probands mother (395A>C) and maternal aunt (395A>G). The maternal grandfather, the mutation origin, shows a nucleotide bi-mutation, carrying the normal and two mutant alleles. Relative to the normal A allele, the C and G mutations in the grandfathers blood leukocytes are about 1/10 and 1/160, respectively. Nucleotide bi-mutations with one allele at such a low frequency would not be commonly detected yet may be frequent. This observation is consistent with a highly mutagenic DNA adduct or lesion present in the zygote. Analysis of F8 and DMD origins shows similar sex ratios of mutation for different mutation types. The origin data from these X-linked genes, in combination, allow a robust appraisal of the circumstances in the human germline under which certain mutational events occur.

**Do spontaneous multiple mutations cluster?** *S. Sommer, J. Wang, K. Hill, X. Li.* Dept Molecular Gen, 2nd Floor, City of Hope, Becker Res Inst, Duarte, CA.

Multiple mutations are sometimes found in patients when all gene regions of likely functional significance are examined. In 1000 consecutive hemophilia B families and 772 independent mutations, three families with two likely causative mutations in the human factor IX gene were identified. Analysis of the mutation origin in two families showed that both mutations occurred in the same germline. Transmission of a second germline mutation is enhanced about 1700-fold relative to the chance occurrence of two mutations. Interestingly, the two doublets involving single nucleotide mutations show closely spaced events (9 and 356 nucleotides separation in a 32 kb region). Similarly, a high rate (about 660-fold) of enhancement is observed for somatic multiple mutations measured by the Big Blue transgenic mouse mutation detection system. The spacing between mutations in lacI mutants is also generally close. The frequency and proximal spacing of spontaneous multiple mutations are consistent with a co-ordinate mutational event. Clustering of multiple mutations was further assayed directly by sequence analysis of an additional 5.3 kb in multiple regions adjacent to the lacI transgenic mutation reporter. Mutants with non-tandem and tandem multiple mutations (66 and 69 mutants, respectively) were examined for additional mutations relative to control mutants with single mutations (135 singlets). Ten additional mutations were identified in six doublets/multipliants, two were found in 66 tandem mutations, while none was found in 135 singlet mutants. Three of the mutants have multiple clusters of mutations: mutant one, a 4 bp new cluster 1477 bp from the original 177 bp cluster; mutant two, a new 113 bp cluster 1411 bp from the original 2 bp cluster; and mutant three, a new 76 bp cluster 1993 bp from an original 391 bp cluster (i.e., there are clusters of mutation clusters). A subset of closely spaced doublets has additional doublets. More sequence is being analyzed. These observations are consistent with the co-ordinate occurrence of spontaneous multiple mutations by a mammalian polymerase that is innately error-prone or in a transient error-prone condition.

**Moving Pharmacogenetics to the Forefront of Modern Medicine: Identifying the Players and Solving the Bottlenecks.** *F.W. Frueh*. Managing Partner, Stepoutside Consulting, LLC, Gaithersburg, MD.

Every year, more than 100,000 deaths occur in the United States that can be attributed to severe adverse drug reactions. Associated with these events are annual health care costs of approximately \$200 billion. At the same time, individualized medicine promises a safer and more effective drug therapy based on the identification and evaluation of key genetic factors present in the patients genome. Even though pharmacogenetics, touted to deliver personalized medicine, has progressed from academic laboratories to large pharmaceutical companies, the research has not yet reached the consumer. The question therefore remains: Why is pharmacogenetics not moving more quickly to the forefront of modern medicine?

In this report, we identify and characterize the players and the steps in the process to illustrate the challenges that need to be overcome and to propose solutions. The key players include (1) the biotechnology industry, including bioinformatics and toolmakers, (2) the pharmaceutical industry, (3) regulators, (4) health care providers, (5) hospitals and physicians, and (6) the patients. Factors to be addressed include standardization of tests, guidelines created by regulators and legislature including the identification and elimination of ethical issues, and the education of physicians and patients. For example, important criteria for an economically feasible introduction of pharmacogenetic testing include the scientific merit and predictive value of the test, the price per test, and the reimbursement through health care providers. The pharmaceutical industry is using pharmacogenetics in the drug development process since a number of years, but without guidelines on how to submit such data to the regulators. The recent announcement by the FDA to release guidance on pharmacogenetics is generally welcomed and a step towards technology standardization and encouragement for the pharmaceutical industry to embrace the challenge for developing better and safer drugs. This and other examples are presented and criteria for each of the key players are identified, prioritized and evaluated.

**Genotyping of single nucleotide polymorphisms by single-base extension and separation by denaturing high performance liquid chromatography.** *P. Chen*<sup>1</sup>, *E.K. Kohlmeir*<sup>1</sup>, *E.H. Cook*<sup>1,2</sup>, *S. Das*<sup>1</sup>. 1) Human Genetics, University of Chicago, Chicago, IL; 2) Psychiatry, University of Chicago, Chicago, IL.

The number of methodologies for the genotyping of single nucleotide polymorphisms (SNPs) has exploded in recent years. While several different methodologies for SNP genotyping exist, no single methodology has been effectively used for all SNPs assayed. We have been using and developing single-base extension in combination with denaturing high performance liquid chromatography (SBE-DHPLC) for the genotyping of several pharmacogenetically relevant SNPs in the field of anti-cancer agents. To date, we have genotyped twenty different SNPs by SBE-DHPLC in the following genes: *CES2*, *UGT1A1*, *UGT2B7*, *MDR1*, *VitD receptor*, *RFC*, *GSTP1*, *MTHFR*, and *HNF-1*. In order to increase the efficiency and throughput of the SBE-DHPLC technique, we have developed duplex PCR and SBE reactions for several of the SNPs genotyped. We have most recently performed a pooled analysis of SBE products allowing successful genotyping of three SNPs in the *CES2* gene simultaneously, per sample. We are currently working on genotyping 4-5 SNPs simultaneously by SBE-DHPLC for novel SNPs identified in the *UGT2B7* gene. The SBE-DHPLC method of genotyping, while not suited for large-scale genotyping, is very well suited for small and medium-scale genotyping projects (up to ~500 samples) and the ability to perform multiplex SBE-DHPLC will result in an increased throughput and decreased cost per genotype. An advantage of the SBE-DHPLC method over other genotyping methods that use labeled primers is the ability to genotype accurately SNPs that contain more than two different alleles. This was important for a SNP in the *MDR1* gene at nucleotide position 2677 where three different alleles were detected. The greatest advantage of the SBE-DHPLC method in our experience is the robustness of the assay and its successful use for genotyping of all conventional SNPs tested in our laboratory to date. Results of our data will be presented and factors important for genotyping by multiplex SBE-DHPLC will be discussed.

Program Nr: 1585 from 2003 ASHG Annual Meeting

**A whole genome amplification strategy for pharmacogenetic studies.** *S. Marsh, C.M. Rose, H.L. McLeod.* Molecular Oncology, Washington University, St Louis, MO.

The recent explosion of information in publicly available polymorphism databases, and the accompanying technology for high throughput genotyping, is a major boon for pharmacogenetic studies. Analysis of single nucleotide polymorphisms with the goal of identifying markers for drug response is no longer limited by the availability of information, rather the finite supply of patient DNA samples. We have used degenerate oligonucleotide primed PCR (DOP-PCR) to prolong the life-span of clinical samples. Using the original DOP-PCR method we assessed 14 different polymorphisms by Pyrosequencing from 12 different chromosomal locations on 10 different chromosomes. With 1ng of DNA there was an overall 7% failure rate of the genotyping assays. Previous studies have found DOP-PCR techniques to over and under-represent areas of the genome. A novel DOP-PCR method using 3 degenerate primers to improve genome representation has recently been described. For each sample, three 20ul DOP-PCR reactions are carried out using three different DOP-PCR primers and 0.5-1ng of DNA. PCR products from the three reactions are pooled. Using this method we assayed markers of pharmacogenetic significance using Pyrosequencing. In addition, Pyrosequencing was carried out on the same samples prior to DOP-PCR amplification. 100% concordance was seen for both the pass rate and results for all samples analyzed. DOP-PCR is a reliable method to prolong the use of clinical samples. Using three different degenerate primers allows for greater genome representation. This technique is highly adaptable to all sources of genomic DNA, including DNA extracted from blood, serum, plasma and fixed tissues.

**Construction of a physical map for human-chimpanzee Y chromosome comparative analysis.** *Y. Kuroki<sup>1</sup>, T.D. Taylor<sup>1</sup>, A. Toyoda<sup>1</sup>, H. Watanabe<sup>1,2</sup>, T. Yamada<sup>3</sup>, S. Morishita<sup>3</sup>, T. Itoh<sup>4</sup>, M. Hattori<sup>1,5</sup>, Y. Sakaki<sup>1,3</sup>, A. Fujiyama<sup>1,6</sup>.*  
1) RIKEN Genomic Sciences Center, Yokohama, Japan; 2) Nara Institute of Science and Technology, Nara, Japan; 3) University of Tokyo, Tokyo, Japan; 4) Mitsubishi Research Institute Inc., Tokyo, Japan; 5) Kitasato University, Sagamihara, Japan; 6) National Institute of Informatics, Tokyo, Japan.

The completion of the human genome sequence allows us to compare genome sequences between human and other species. Comparative genome analysis of human and its closest relative, the chimpanzee, will reveal critical regions which are conserved between the two species or are species-specific. Previously, we have made a human-chimpanzee whole genome comparative analysis\* to begin to understand human-specific characteristics. This study, which placed 52,881 chimpanzee BAC clones on the whole human genome, showed that the degree of conservation of the Y chromosome sequence in human-chimpanzee was much lower in comparison to the other chromosomes.

To address the question of lower coverage of the Y chromosome, we have started comparative analysis between human and chimpanzee Y chromosomes. By using the human Y chromosome sequence, we have designed 300 STSs and screened them against both genomes. The results revealed that some of the male-specific STSs showed human-specific amplification. We are constructing the chimpanzee Y chromosome map from a whole-genome BAC library and a Y-specific fosmid library, both of which were made from a single male chimpanzee. The chimpanzee Y chromosome-specific fosmid library is estimated to cover 20X of the chimpanzee Y chromosome. Furthermore, the sequencing of 800 chimpanzee Y fosmid clone ends is currently in progress. In this analysis we have found that some fosmid clones do not map to any human chromosomes by FISH, although they clearly map to the chimpanzee Y chromosome. This indicates that these clones contain sequences specific to the chimpanzee Y chromosome.

\*Fujiyama et al. *Science*, 295, 131-134, 2002.

**Fine Mapping of Genes Deleted on Mouse Chromosome 18 in Hepatocellular Carcinoma, and Relationship to Human Chromosome 5q.** *J. Hozier*<sup>1,2</sup>, *L. Davis*<sup>2,3</sup>. 1) Dept. Pathology, Univ New Mexico, ABQ, NM; 2) Applied Genetics Labs, Inc. Mellbourne, FL; 3) Exagen Corp., ABQ, NM.

The B6C3F1 mouse is used worldwide to gauge the carcinogenic hazard posed to humans by chemical agents. Evaluation of this model for predicting human neoplasia requires a comparison of the genetics of tumor development between the two species. In previous work we used simple sequence repeat (SSR) loci to find regions of loss of heterozygosity (LOH) that might be associated with tumor suppressor gene function in liver tumors of B6C3F1. In that study we found recurrent LOH associated with hepatocellular carcinoma (HCC) on chrs. 2,5,8,9,14 and 18. In the study described here we have determined the critical region on chromosome 18 using a denser set of 14 SSR markers. We analyzed the chr.18 LOH region for frequency and size of deletions, gene content, and the orthologous genomic region on human chromosome 5q, and found the following: 1.LOH on mouse chr.18 occurs in about 30% of the tumors studied with the denser SSR set, compared to 3.5% found with the original set of chr.18 SSR markers. That is, most LOH events on mouse chr.18 are subchromosomal. 2.The minimum size of the LOH as measured with the denser SSR marker set is about 1.4 Mb. 3.This region of mouse chr.18 contains 5 known genes and a small number of relatively uncharacterized expressed sequences, which become candidates for tumor suppressor loci in mouse HCC. 4.By comparing the mouse chr.18 LOH region with the orthologous region on human chromosome 5q, using the comparative mapping functions available in the Golden Path (UCSD) and Ensembl genomic databases, we find that the minimal region of LOH on mouse chr.18 has been conserved intact in the human genome, but an evolutionary breakpoint has occurred within about 100kb of one end of this region, placing the HCC minimal region in a different genetic environment in the human (relative to mouse) genome. In the context of loss of tumor suppressor gene function caused by deletion of fairly large regions of the genome, deletions associated with particular tumors may affect different sets of genes in two species due to evolutionary shifts in the linear organization of genes.

**A Novel Method for Molecular Haplotyping Combining an Improved AS-PCR Technique with Base Specific Cleavage of Nucleic Acid Analyzed by Mass Spectrometry.** *M. Beaulieu, D. Kosman, M.R. Nelson, D. van den Boom.* Sequenom Inc., San Diego, CA.

Haplotypes, the units of inheritance, can provide additional power in detecting disease association. Here we describe a novel approach that enables direct molecular haplotyping of multiple polymorphic markers in a single reaction. To perform haplotyping the chromosomes must be isolated prior to genotype analysis. Allele-Specific PCR (AS-PCR) is a popular method to selectively amplify a locus from the desired allele/chromosome from which genotypes data can be inferred into haplotypes. However, available AS-PCR methods are often leaky, leading to ambiguous results. We have developed a novel AS-PCR technique to improve specificity. A non-extendable and exonuclease resistant competitor oligonucleotide is added to the reaction. The latter feature allows the use of proofreading polymerases permitting long-range AS-PCR. We called this novel strategy: Non-Extendable Exonuclease Resistant AS-PCR (NEER-AS-PCR). The amplicons can be used as template for genotyping assays. We have used quantitative base extension reactions, using MassEXTEND, and demonstrated significant reduction in leakage when using 10-fold excess of the competitor. Regions from the CETP gene locus were analyzed to assess accuracy. For multiple marker haplotypes, markers usually must be genotyped individually. To circumvent this issue we combined NEER-AS-PCR with base-specific cleavage of nucleic acid (MassCLEAVE method). The NEER-AS-PCR product was subject to in vitro transcription and cleaved in four reactions. The cleavage products were analyzed by MALDI-TOF MS generating sequence specific mass signal patterns. Cross-comparison of theoretical and experimental mass pattern allowed haplotype determination. We applied this method to the determination of molecular haplotypes in the CETP gene and provide a comparison to haplotypes inferred from individual genotyping. Because the analysis is not exclusive to known markers, novel variations associated with sub-haplotypes were discovered. Combined with MALDI-TOF MS this method allows the generation of comprehensive haplotype information at un-precedented speed and accuracy.

**A multiplexed ultra-high throughput genotyping platform based on the oligonucleotide ligation assay: Design pipeline, conversion rate, and genotyping accuracy.** *J.P. Day<sup>1</sup>, R.T. Koehler<sup>1</sup>, N. Peyret<sup>1</sup>, C. Scafe<sup>1</sup>, R. Lippert<sup>1</sup>, M. Rhodes<sup>1</sup>, J. Ziegle<sup>1</sup>, K.C. Hayashibara<sup>1</sup>, C. Chen<sup>1</sup>, M. Wenz<sup>1</sup>, K. Hennessy<sup>1</sup>, E.G. Spier<sup>1</sup>, D. Gordon<sup>2</sup>, F.M. De La Vega<sup>1</sup>.* 1) Applied Biosystems, Foster City, CA; 2) Rockefeller University, NY, NY.

The feasibility of whole-genome genetic association studies requires the ability to accurately genotype hundreds of thousands of SNPs in thousands of samples at a reasonable cost. Applied Biosystems has developed the SNPLex System; a multiplexed ultra-high throughput genotyping platform based on the oligonucleotide ligation assay (OLA) which utilizes capillary electrophoresis as the detector. This platform has several advantages: the OLA has been previously shown to be an accurate and robust assay which requires only a small amount of genomic DNA samples; no locus-specific PCR is required; multiplex levels of up to 96 assays per tube are expected on the first generation; and the endpoint readout in a Applied Biosystems 3730xl 96 capillary electrophoresis instrument can resolve 9,216 genotypes per 15 min run, or over 500,000 genotypes per day. Since high-throughput capillary electrophoresis instruments are common in laboratory settings for use in sequencing applications, the technology adoption is very fast and cost effective. An algorithm pipeline was developed for the design of the assays, including the OLA probe designs and the multiplex pooling scheme. Steps are taken to identify and remove SNPs in repetitive and duplicated regions, to select the optimal strand to target probes, and to avoid deleterious probe interactions and spurious ligation templates. Statistics of the in silico assay design, successful assay conversion, and genotype call rates are presented. Using as a baseline for comparison the genotypes from high quality dideoxy sequencing allele calls (over 1,000 SNPs with genotypes in at least 14 samples), the accuracy and genotype misclassification rates of the 5 nuclease and the SNPLex assays are compared.

**Improving the odds in comparative sequence analysis Mass spectrometry for sensitive detection of sequence variations.** *C. Honisch<sup>1</sup>, M. Ehrich<sup>1</sup>, N. Storm<sup>2</sup>, S. Bocker<sup>3</sup>, M.R. Nelson<sup>1</sup>, P. Stanssens<sup>4</sup>, M. Zabeau<sup>4</sup>, D. van den Boom<sup>1</sup>.* 1) SEQUENOM Inc., 3595 John Hopkins Court, San Diego, CA 92121,USA; 2) SEQUENOM GmbH, Mendelssohnstr. 15d, 22761 Hamburg, Germany; 3) Technische Fakultaet Universitaet Bielefeld, AG Genominformatik, Bielefeld, Germany; 4) Methexis Genomics NV, Technologiepark 4, B-9052 Zwijnaarde, Belgium.

With the sequencing phase of the Human Genome Project completed, researchers have been stepping into the next endeavour of elucidating the genetic code and its interindividual variation.

We introduce a novel scheme for high-throughput comparative sequence analysis by MALDI-TOF mass spectrometry.

A single-stranded copy of a PCR amplified target sequence of interest is generated by in vitro transcription and cleaved in four reactions at positions corresponding to each of the four bases. The cleavage products are analysed by MALDI-TOF mass spectrometry generating four sequence specific mass signal patterns.

Reference sequences are used to construct an in silico cleavage pattern enabling cross-comparison of theoretical and experimental mass patterns. Pattern identity results in sequence validation. Pattern deviations in form of additional and missing signals allow for the detection, identification and localization of sequence changes. Data of the four separate cleavage reactions substantiate redundancy and reliability of results.

The combination of mass spectrometry with base-specific cleavage offers significant advantages in speed and accuracy of data acquisition.

The expansion of the portfolio of available molecular methods by base-specific cleavage and mass spectrometry is a significant milestone in the field of comparative genomics and genetics and opens new routes in the detection of epigenetic modifications - DNA methylation analysis. Mutation detection and pathogen identification are additional fields of application.

Program Nr: 1591 from 2003 ASHG Annual Meeting

**Construction of SNP marker sets for genome-wide association studies.** *G.E. Marnellos, S. Kammerer, M.R. Nelson, C.R. Hoyal, A. Braun.* Sequenom Inc., San Diego, CA.

DNA sequence variations between individuals are an important tool for identifying genetic contributions to disease. Single nucleotide polymorphisms (SNPs), with their dense distribution across the genome, are excellent markers for association studies. We have created working assays for over 200,000 publicly available putative SNPs in gene regions and tested them on pooled DNA samples from 94 CEPH Caucasians with a method based on primer extension and MALDI-TOF mass spectrometry. More than 100,000 SNPs mapping uniquely onto the genome were found to be polymorphic with minor allele frequency greater than 5%. These polymorphic SNPs cover the genome with a density that reflects the distribution of genes.

In order to create sets of SNP markers for genome-wide association studies, we developed an algorithm to select subsets of these polymorphic SNPs with more regular spacing and satisfying other criteria, such as proximity to genes, location in coding versus non-coding regions, and high minor allele frequency. The algorithm sets up regularly spaced posts at a specified density across the genome, identifies the SNPs within each post neighborhood, ranks them by the above criteria and by their distance to the post, and picks the top-ranking SNP in each neighborhood. Using this algorithm we have selected sets of about 25,000 and 50,000 SNPs located within 10Kb of more than 65% of known and predicted genes. These marker sets have been used successfully in 12 genome-wide scans for common diseases and quantitative traits, including breast, lung and prostate cancers, type II diabetes, hypertension and HDL-cholesterol levels.

As more public SNPs and annotation become available, we are using the algorithm to update and expand our SNP sets and fill gaps as needed, focusing primarily on gene regions; the goal is to have a SNP every 10Kb within all currently known and predicted genes. For SNPs not yet validated, our algorithm selects SNPs with features we have found to predict polymorphic SNPs. When haplotype maps become available, that information will also be used to select the most informative SNPs for genome-wide association.

**Association of the 4qA allele with Facioscapulohumeral Muscular Dystrophy (FSHD) in British and Turkish Populations.** *M. Upadhyaya*<sup>1</sup>, *K. Wiseman*<sup>1</sup>, *P. Serdaroglu*<sup>2</sup>, *M. MacDonald*<sup>1</sup>. 1) Dept Medical Genetics, Univ Wales Col Medicine, Cardiff, UK; 2) University of Istanbul, Istanbul, Turkey.

Facioscapulohumeral muscular dystrophy is an autosomal dominant disorder affecting one in 20,000 and is caused by the deletion of D4Z4 repeat units located to 4q35. Recently a polymorphism in the -satellite repeats distal to the D4Z4 repeat units on chromosome 4, with two allelic forms (4qA and 4qB), has been identified. Allele 4qA was found to co-segregate with FSHD-specific short D4Z4 alleles in the Dutch population. In the normal population, both polymorphic alleles (4qA/4qB) are present in equal frequency. In order to ascertain whether the 4qA allele segregates with the small FSHD allele in different populations, we have studied a panel of 65 unrelated FSHD individuals, of whom 50 were British and 15 were of Turkish origin. Fifty unaffected individuals of mixed origin were also examined as controls. High molecular weight DNA was extracted from blood samples, and p13E-11 short fragments were identified by digestion with the restriction enzymes *EcoRI*, *XapI* and double digestion with *EcoRI* and *BlnI*. DNA was resolved using pulsed field gel electrophoresis. The Southern blots were hybridised with radiolabelled 13E11 probe and visualised by autoradiography. To study the distribution of alleles 4qA and 4qB, DNA samples were digested with the restriction enzyme *HindIII*, and the Southern blots were hybridised consecutively with probes specifically designed to illuminate alleles 4qA and 4qB (probes A and B). In all 65 affected individuals, allele 4qA was always found to segregate with the FSHD specific small fragments identified with the probe 13E-11. In contrast an equal frequency of alleles 4qA and 4qB was observed in the un-affected control individuals. This study confirms the specific association of the allele 4qA with the FSHD disease allele in both British and Turkish populations. This study not only contributes to the molecular biology of FSHD but will also refine the molecular diagnosis for FSHD in the patients with borderline short *EcoRI/BlnI* fragments. These findings further emphasise that allele 4qA may well have some functional relevance in the pathophysiology of FSHD.

**Detection of gene variants utilizing Surveyor CEL Nuclease Detection Technology and various DNA separation platforms.** *J.G. Rudolph III<sup>1</sup>, J. Durocher<sup>1</sup>, J. Breen<sup>1</sup>, B. Walters<sup>1</sup>, P. Qiu<sup>2</sup>, H. Shandilya<sup>2</sup>, J.M. D'Alessio<sup>2</sup>, S. Lilleberg<sup>1</sup>, G.F. Gerard<sup>2</sup>.* 1) Biosciences Discovery Services, Transgenomic, Inc, Gaithersburg, MD; 2) Research and Development Transgenomic, Inc, Gaithersburg, MD.

The discovery of mutations and SNPs within the human genome is a crucial step towards the elucidation of both inherited and spontaneous diseases ranging from schizophrenia to cancer. Identifying genetic variants will provide a better understanding of the underlying causes of specific diseases and, consequently, novel and improved treatments are likely to be discovered. DHPLC (denaturing high performance liquid chromatography) technology has always been an efficient and useful tool for screening candidate genes for these deleterious mutations. There are certain gene sequences however, due to their sequence content, which do not lend themselves to accurate and efficient DHPLC screening. We have developed a fast and efficient alternative known as Surveyor CEL Nuclease Mutation Detection Technology. This enzymatic method is highly sensitive and can be used to detect both known and unknown mutations including all base substitutions as well as insertions/deletions up to at least 12 bases. In addition, there are no limitations placed on the DNA fragment being analyzed, either in total length, position of the mutation, or sequence content of the fragment and multiple mutations within the same fragment can be detected. This system is based on a new class of mismatch specific endonucleases, called CEL Nuclease, which can be used with a variety of DNA detection platforms including the WAVE, manual gel electrophoresis, or automated gel or capillary electrophoresis platforms. The detection of mutations in a number of genes including APC, Kras, and -tubulin, with genomic DNA obtained from a variety of sources including cell lines, human tissue, and lymphocytes is compared. We find that the results obtained using Surveyor correlate closely with those obtained using DHPLC alone, and in some circumstances can be used in a wider variety of situations compared to DHPLC.

Program Nr: 1594 from 2003 ASHG Annual Meeting

**Determination of Y-Haplogroups Utilizing Suspension Arrays.** *D. Carlson, C. Rowe, Z. D'Aoust.* Marligen Biosciences, Inc, Ijamsville, MD.

The purpose of this study was to develop a method for rapid determination of Y-haplogroup of DNA samples. Forty-two Y-chromosome single nucleotide polymorphisms (SNPs) were selected based on their association with a particular ancestral haplogroup. Each polymorphic region was amplified in a multiplexed PCR reaction utilizing primers labeled with a fluorescent reporter dye. Oligonucleotides specific for each allele in the multiplex were immobilized on the surface of latex microspheres containing fluorescent identifiers. Labeled PCR products were then hybridized to the microspheres in multiplexed mode. Reporter dyes and identifier dyes were read simultaneously for each microsphere in a flow analyzer. Allele-calling software (MasterPlexGT, MiraiBio) automated the genotyping. The flow reader required 1.5 hr to analyze 96 wells of multiplexed reactions. The most frequent Y-haplogroups were found to be E1a for African Americans and R1b for U.S. Caucasians. The percentage of admixture of Caucasian Y-haplogroups in the African American samples was slightly more than 30%. Frequency of African markers in the Caucasian samples was less than 5%.

**The UV wavelength-specific role of p53 in transcription-coupled nucleotide excision repair is not through differential binding of the p53 protein to its consensus sequence of downstream effectors such as p21, GADD45, XPC and p48-XPE genes.** *W. Dridi<sup>1</sup>, R. Fetni<sup>2</sup>, E.A. Drobetsky<sup>3</sup>, R. Drouin<sup>1</sup>.* 1) Medical Biol, Hosp St Francois, Laval Univ, Quebec, PQ, Canada; 2) Cytogenetics Montreal Children's Hospital, Montreal, Quebec, Canada; 3) Guy-Bernier Research Center, Maisonneuve-Rosemont Hospital, Montreal, Quebec, Canada.

**Introduction:** In response to UV, p53 degradation is decreased and p53 transcription is activated.. It is also well established that this tumor suppressor regulates nucleotide excision repair (NER) of cyclobutane pyrimidine dimers (CPD), the main UV-induced DNA photoproduct. The role of p53 in transcription-coupled NER (TCNER, a sub-pathway of NER) is UV wavelength dependent (Mathonnet G et al. PNAS 100:7219, 2003), since functional p53 is required for TCNER after irradiation with polychromatic UVB (280-320-nm) but not after 254-nm UVC. **Objective:** We aimed to investigate the mechanism underlying this UV wavelength specificity by using an in vivo footprinting approach in order to determine the kinetics of p53 binding to its consensus sequence along the promoters of downstream effectors such as p21, GADD45, XPC and p48-XPE genes following either UVC or UVB irradiation. **Methods:** Primary human fibroblasts were irradiated with low doses of UVB or UVC. At different time points (0, 60, 120, 240 and 480 min), cells were treated with either one of the three footprinting agents (dimethylsulfate, UVC and DNaseI were used), DNA was purified, and ligation-mediated PCR was carried out for the p21, GADD45, XPC and p48-XPE gene promoters. **Results:** The presence of footprints confirmed the binding of p53 to its consensus sequence at all promoters examined, however no differences were noted in this respect following UVB vs. UVC irradiation. **Conclusion:** The UV wavelength-dependency for p53 in TCNER does not involve differential DNA binding kinetics of p53 to its consensus sequence along the promoters of critical downstream effectors.

**High throughput digital typing of human-specific L1 insertions.** *R.M. Badge*<sup>1</sup>, *J.V. Moran*<sup>2</sup>. 1) Genetics, University of Leicester, Leicester, UK; 2) Human Genetics, University of Michigan Medical School, Ann Arbor, USA.

ATLAS (amplification typing of L1 active subfamilies) is a genomic screening method that selectively amplifies the termini of young L1s and their flanking genomic sequences. Primers directed to active L1 subfamily-specific sequence variants ensures strong enrichment. High resolution gel electrophoresis enables the display of hundreds of amplicons that can be identified by PCR isolation and sequencing. Previously we demonstrated (*Badge et al*, 2003) that ATLAS can successfully identify full length novel (i.e. absent from the reference human genome sequence) human-specific L1s some of which are able to retrotranspose at high frequency in cell culture. One of the characterised insertions, obtained from a screen of only ten unrelated individuals, was apparently private- it was restricted to the individual in which it was discovered. This suggests that such insertions are much more common than previously suspected and that typing of many such insertions could be an effective individual identification method. By combining ATLAS and sequential oligonucleotide hybridisation we have developed a high throughput digital typing method which allows ongoing analysis of novel L1 insertions as they are identified. We present a pilot study on the efficacy of this method by comparison with genotyping data obtained by traditional PCR typing assays. The method is simple, rapid and extensible, and takes advantage of the largely unique sequences generated at the insertion points of full-length human-specific L1 retrotransposons. We demonstrate that it is also possible to "invert" the assay by immobilising the insertion-specific oligonucleotides as a macro or microarray, enabling the simultaneous typing of many loci in a given individual. In addition since the amplicons selectively enriched by ATLAS are short (100-1000bp) DNA integrity is not critical. As a result we have investigated the use of isothermal whole genome amplification to generate templates for ATLAS, potentially allowing the systems application to forensic samples. *Badge RM, Alisch RS, Moran JV. ATLAS: a system to selectively identify human-specific L1 insertions. Am J Hum Genet. 2003 Apr;72(4):823-38.*

**Use of the L1 retrotransposon for functional genomics.** *E.M. Ostertag, J.L. Goodier, J.M. Choi, D.V. Babushok, A.H. Farley, H.H. Kazazian.* Dept. of Genet., U. of Pennsylvania, Philadelphia, PAA.

Previously we described a mouse model of human L1 retrotransposition in which L1 elements retrotranspose in male meiosis. In the most active transgenic line the new insertion rate was estimated by breeding to be 1 in 68 sperm. We had sequenced two insertions and found that they had all the characteristics of endogenous L1 insertions. We have now improved the retrotransposition estimate in this line to 1 in 40 sperm by finding insertions in 5 offspring among 200 tested. Recently, we developed new transgenic lines that had the same L1 element and heterologous promoter. In order to estimate the retrotransposition frequency in these mice without cumbersome breeding, we developed a quantitative PCR method on sperm DNA sufficiently sensitive to detect one retrotransposition event in 1000 sperm. Of 15 new transgenic lines, we found 3 (20%) with retrotransposition frequencies 2-4 fold greater than the previously studied line, or 1 insertion in every 10-20 sperm. Knowing that we can readily obtain transgenic lines with substantial retrotransposition frequencies, we hypothesized that L1 elements would be ideal for use in random mutagenesis. In addition to a high rate of insertions, L1s insert randomly and stably throughout the genome. Importantly, mutagenesis using L1s occurs in the germline without the need to proceed through embryonic stem cells. Therefore, we developed a bi-directional splice acceptor gene trap and showed that it does not interfere with L1 retrotransposition in cultured cells. Using Thermally Asymmetric Interlaced-PCR (TAIL-PCR), we were able to rapidly map 22 of 27 new inserts (81%) in cultured cells. With these new advances, we are proceeding to create new transgenic lines using 1) improved promoters to boost L1 expression in meiosis, 2) a newly-discovered human L1 that is 50% more active than the previously tested element, and 3) a new gene trap cassette to increase the detection rate of new insertions. In the near future, we expect to obtain transgenic lines with one gene trapped in every 5-10 sperm. Since these lines will allow production of gene knockouts by natural reproduction, they should be very useful for mouse functional genomics.

**Evolution of LINEs and great apes genomes.** *G.D. Swergold, S. Chi, M. Ulz, L. Mathews.* Department of Medicine, Columbia University, New York, NY.

Comparisons of gene sequences reveal a high level of conservation between humans and the other great apes despite the existence of large physiological and behavioral differences. This suggests that other types of genomic change may account for some of the phenotypic differences and may have played an important role in the evolution of our species. LINE-1 (L1) retrotransposons have been amplifying throughout the mammalian radiation and comprise ~17% of the human genome but little is known about their biology in the other great apes. We report our investigations into the role of L1s in the evolution of great ape genomes. PCR genotyping of L1PA2-6 insertions in great apes and old world monkeys established the periods of amplification of these subfamilies during great ape evolution. A molecular method (LOAF library construction) was developed to sample great ape genomes for recent L1 insertions. From the proportion of lineage-specific insertions in the libraries we determined that the accumulation of full-length L1s has been 2-3 fold more rapid in *Pan* than in *Homo* since their divergence. We identified and fully sequenced 4 chimpanzee, 5 gorilla and 6 orangutan lineage-specific insertions. One gorilla insertion was polymorphic in the modern lowland gorilla population. A molecular phylogeny constructed using these sequences and a large number of human-specific and shared subfamily L1 insertions indicates that most are quite young and may retain retrotransposition ability. Based on the sequence alignment (and confirmed by PCR amplifications using sequence shared variants) we describe specific L1 subfamilies for each of the major non-human great ape lineages. Phylogenetic analysis also revealed that a large majority of human-specific and *Pan*-specific L1 insertions, as well as a gorilla-specific L1 subfamily descended from a subset of L1PA2 elements. Our data also suggest that certain older insertions that to date have been classified as separate subfamilies may not be distinct evolutionary units. Our results provide a framework for understanding the molecular evolution of L1s in the great apes and their impact upon the evolution of their host species.

**Expansion of GAA triplet-repeats in the human genome: A ticking bomb?** *R.M. Clark*<sup>1</sup>, *G.L. Dalgliesh*<sup>1</sup>, *D. Endres*<sup>2</sup>, *S.I. Bidichandani*<sup>1</sup>. 1) Dept Biochem & Molec Biol, Univ Oklahoma Health Sci Ctr, Oklahoma City, OK; 2) Math & Stats, Univ of Central Oklahoma, Oklahoma City, OK.

Friedreich ataxia is caused by expansion of a GAA triplet-repeat (GAA-TR) in the *FRDA* gene. Normal alleles contain <30 triplets, and disease-causing expansions (66-1700 triplets) arise via hyperexpansion of premutations (30-65 triplets). To gain insight into GAA-TR instability we analyzed all triplet-repeats in the human genome. We identified 988 (GAA)<sub>8+</sub> repeats, 291 with 20 triplets, including 29 potential premutations (30-62 triplets). To compare the observed size distribution of GAA-TR sequences we extended our study to include all other nine possible triplet-repeat motifs. We found that most triplet-repeats were restricted in length to <20 triplets and that GAA-TR sequences were clearly the longest. The reason for the elongation of GAA-TR sequences in the human genome is presently unclear. A theoretical estimation of the expected maximum length for GAA-TR sequences in the human genome indicated that (GAA)<sub>6+</sub> sequences should be extremely rare. Analysis of the flanking sequence showed that 89% of (GAA)<sub>8+</sub> sequences have arisen within large G/A-rich islands, and 58% map within poly-A tails of *Alu* elements. GAA-TR sequences located within evolutionarily old *Alu* J elements were significantly longer than those within younger *Alu* elements, indicating that they have undergone continued expansion throughout primate genomic evolution. Over 40% of the (GAA)<sub>8+</sub> sequences, including five potential premutations with >30 triplets mapped within genes (mostly introns, some in UTR sequence). We identified at least one other GAA-TR sequence that has undergone a similar multi-step expansion process, displaying normal, premutation and expanded alleles as seen at the *FRDA* locus. Therefore, GAA-TR expansions analogous to Friedreich ataxia mutations are not likely to be limited to the *FRDA* locus. These data indicate that GAA-TR sequences have a significant predisposition for undergoing expansions. They have undergone continued expansion throughout primate evolution, resulting in several potentially mutagenic tracts, many of which map within genes and may therefore be potentially disease-causing.

**Defining 6p25 segmental duplication and deletion breakpoints with a high-resolution genomic microarray.** *O.J. Lehmann*<sup>1,3</sup>, *D.M. Beare*<sup>2</sup>, *H. Fiegler*<sup>2</sup>, *C.F. Langford*<sup>2</sup>, *P.T. Khaw*<sup>1,3</sup>, *N.P. Carter*<sup>2</sup>, *I. Dunham*<sup>2</sup>, *S.S. Bhattacharya*<sup>1</sup>, *A.J. Mungall*<sup>2</sup>. 1) Dept Molecular Genetics, Institute of Ophthalmology, London, UK; 2) Wellcome Trust Sanger Institute, Cambridge, UK; 3) Moorfields Eye Hospital, London, UK.

Mutations and dosage changes in *FOXC1* result in ocular and non-ocular developmental anomalies as well as early-onset glaucoma. The existence of 6p25 segmental duplications and deletions encompassing *FOXC1* in multiple unrelated pedigrees, indicates that a common mechanism mediates these chromosomal rearrangements. Having previously determined the approximate extent of these segmental duplications/deletions with microsatellite marker genotyping and fluorescent *in situ* hybridisation, we have constructed a high-resolution genomic microarray extending over 680kb of 6p25, a region that includes the duplicated interval. This tiling path of 544 individual PCR products (mean size 1.25 kb) is being arrayed onto amine-binding slides. The (tiling path) arrays will be interrogated with DNA samples from 5 pedigrees with 6p25 chromosomal anomalies (4 segmental duplications, 1 segmental deletion) in which the breakpoints have been localised to single bacterial artificial chromosome clones. It is anticipated that array analysis will define the position of these breakpoints to regions of less than 5kb, and potentially to within a single PCR product, allowing the sequences mediating these rearrangements to be identified.

**RPGR gene mutations cluster upstream of a degenerated coding polypurine/polypyrimidine minisatellite in exon ORF15.** *I. Bader*<sup>1</sup>, *M. Hergersberg*<sup>2</sup>, *A. Meindl*<sup>3</sup>, *T. Meitinger*<sup>1</sup>. 1) Inst Human Genetics, GSF-Natl Research Ctr, Munich-Neuherberg, Germany; 2) Kantonsspital Aarau, Switzerland; 3) Department of Medical Genetics at the Ludwig-Maximilians-University Munich, Germany.

Mutations in the X-chromosomal RPGR gene account for up to 20% of all cases of retinitis pigmentosa, a figure higher than for any other single locus of the more than 20 different RP loci mapped throughout the genome. This high degree of sequence variation is due to a large 3terminal exon (ORF15) of an alternatively spliced transcript predominantly expressed in retina. It contains a repetitive purine-rich sequence coding for an acidic tail (>250 glutamic acid residues). We performed dot-matrix, tandem-repeat and PCR analyses of the RPGR genomic sequence. Comparative analyses in mouse, cattle and pufferfish indicate that the polypurine/polypyrimidine sequence of exon ORF15 shares features of a minisatellite in all species. These features include a tandem array with repetitive motives ranging in length from 18 bp (*Mus Musculus*) to 39 bp (*Fugu rubripes*) and a length variability of the array detected in control chromosomes of man, mouse and cattle. In addition we looked at the spectrum and distribution of mutations of four comprehensive mutational screens (486 X-linked RP patients, including 58 own cases) and found that the majority of the 106 disease-causing mutations (frameshifts caused by small deletions and nonsense mutations) cluster in the sequence adjacent to the 5' end of the diverged minisatellite. In contrast in-frame sequence variations (~34% of controls) with consequent losses or gains of tandem repetitive units are located throughout the degenerated polypurine/polypyrimidine minisatellite. From this observation we hypothesize the presence of two mutational mechanisms in the human exon ORF15: a) a slipped stranded mispairing mechanism leading to loss or gain of tandem repetitive units and b) a "gratuitous" DNA repair mechanism, triggered by the triplex-formation of the polypurine/polypyrimidine tract inducing enhanced mutagenesis in the flanking sequence.

**The Gene Expression Patterns of Cultured Embryo Rat Cortexes Induced by Hyperphenylalanine.** *X. Gu*<sup>1</sup>, *H.W. Zhang*<sup>2</sup>. 1) Xin Hua Hosp, Shanghai Second Medical University, Shanghai, China; 2) Shanghai Institute for Pediatric Research, ShanghaiChina.

Phenylketonuria is marked by hyperphenylalaninemia and mental retardation, the purpose of this work is to study the expression specturm of cultured embryo rat cortexes induced by hyperphenylalanine and to understand the mechanism of neuronal injury in PKU patients. Cultured embryo rat cortexes for 3 days were randomly assigned to the two groups: hyperphenylalanine and control. Neurons in group hyperphenylalanine were induced by 0.9M hyperphenylalanine for 12 hours. The control group was treated with same volume of medium. Total RNA was extracted and hybridized with the Affymetrix gene chip U34 according to the protocol provided by the Affymetrix Company. Some gene expression results were validated by real-time PCR technique. The expression of 167 probe sets (16,6%) plotted on the chip were increased among the whole of 1323 probe sets, which could be functionally categorized to signal transduction, neuron related, cytoskeleton, metabolism, ion channel, transcription factor, cytokines, apoptosis related and so on. Especially, CaMKRasP38Ca<sup>2+</sup> channel, some genes related to vesical forming, releasing of neurotransmitter, and some glutamate receptors and transportors were up-regulated in the environment of hyperphenylalanine. Seven probe sets were decreased, which account to 0.5% of tested gene. We conclude that the process of neuron injury induced by hyperphenylalanine was multifactorial, hyperphenylalanine could activate the axis of NMDR-Ca<sup>2+</sup>- CaMK- Ras- P38, releasing of neuron transmitter may be abnormal in this condition and neuroexciting transmitter glutamate could take part in the process.

**Identification and detection of the 6.3kb deletion breakpoint in the PAH gene located between L1 and Alu repetitive elements.** *N. Gal<sup>1</sup>, G. Schwartz<sup>1</sup>, R. Loewenthal<sup>2</sup>, Y. Anikster<sup>1</sup>.* 1) Safra children hospital , Sheba Medical Center, Tel Hashomer, ISRAEL; 2) Tissue Typing Unit, Safra Children's Hospital, Sheba Medical Center, Tel-Hashomer, Israel.

Phenylketonuria (PKU), one of the most common autosomal recessive inborn errors of metabolism, is caused by mutations in the gene encoding the hepatic enzyme phenylalanine hydroxylase (PAH). The average incidence worldwide is 1/10,000. However, the incidence varies widely across different geographic areas and ethnic origins. Prevalence is highest in Ireland, western Scotland and among Yemenite Jews (YJ). A founding-father origin of the PKU mutation was found in YJ. Southern-blot analysis of this mutation showed a ~6.7kb deletion spanning the third exon of the PAH gene (Nature, V. 344, p. 168-170, 1990). In order to identify the exact deletion breakpoints we designed PCR primers from the genomic sequence data of the PAH gene (Genebank accession no AF404777). One set of primers amplified an 812bp fragment present only in the deletion alleles. Aligning the breakpoint sequence to the normal genomic sequence revealed a deletion of 6296 bp which included 2317bp of IVS2, 184bp of exon 3 and 3795bp of IVS3. Interestingly, the 5' and 3' breaks occur within the repetitive elements LINE- L1 and SINE-Alu Sx, respectively. This could suggest an explanation of the mechanism involved in the deletion formation. The addition of exon 3 primers (281 bp apart) to the PCR with the deletion primers created a multiplex PCR system, which allows identification of patients with zero, one or two alleles bearing the 6.3kb deletion. This methodology constitutes a significant advance over current detection of the PAH deletion in Yemenite Jews. By using this method we confirmed the high prevalence of the deletion in the YJ community and are able to detect compound heterozygotes in mixed origin families and to offer prenatal diagnosis.

**A Slc7a7 null mouse model of lysinuric protein intolerance.** *M.P. Sperandeo<sup>1,2</sup>, V. Ammendola<sup>1,2</sup>, V. Fiorito<sup>1</sup>, A. Pepe<sup>1</sup>, M. D'Armiento<sup>3</sup>, R. Vecchione<sup>3</sup>, G. Andria<sup>1</sup>, G. Borsani<sup>4</sup>, V. Marigo<sup>5</sup>, S. Banfi<sup>5</sup>, A. Ballabio<sup>5</sup>, G. Sebastio<sup>1</sup>.* 1) Dept. of Pediatrics, Federico II University, Naples, Italy; 2) Dulbecco Telethon Institute, Rome, Italy; 3) Dept. of Pathology, Federico II University, Naples, Italy; 4) Dept. of Biomedical Sciences and Biotechnology, University of Brescia, Italy; 5) Telethon Institute of Genetics and Medicine, Naples, Italy.

Lysinuric protein intolerance (LPI, MIM 222700) is an autosomal recessive defect of cationic amino acids transport at the basolateral membrane of epithelial cells in the intestine and kidney, caused by mutations of the SLC7A7 gene. The unknown pathogenesis of the multisystem involvement of LPI, which includes a life-threatening alveolar proteinosis, and the lack of an effective treatment has prompted the creation of a LPI animal model. A constitutive knockout of *Slc7a7* was generated by random insertional mutagenesis in embryonic stem cells (Lexicon Genetics Inc, Texas). More than 400 *Slc7a7*<sup>+/-</sup> intercrosses led to only one *Slc7a7*<sup>-/-</sup> live male. Since birth, this null animal showed a severe failure to thrive compared to siblings. The null animal, still alive at 12 months of age, shows also a strong reduced fertility compared with <sup>+/-</sup> and <sup>+/+</sup> littermates. It is currently fed on a low protein diet and citrulline supplementation. Five additional *Slc7a7*<sup>-/-</sup> died within the first day of life, all showing severe growth failure. To establish the timing of the apparent lethality of *Slc7a7*<sup>-/-</sup> mice, embryos were collected at E 16.5 and E 18.5 stages, respectively. The proportions of *Slc7a7* genotypes were found as expected for an autosomal recessive transmission. These data suggest that *Slc7a7*<sup>-/-</sup> pups die early in the perinatal period and not during the embryogenesis. Most of *Slc7a7*<sup>-/-</sup> pups were probably lost at birth because of cannibalism. At E 16.5 stage, the *Slc7a7*<sup>-/-</sup> embryos were already smaller than <sup>+/-</sup> or <sup>+/+</sup> sibs. None of the null embryos showed gross morphological abnormalities. In humans, LPI does not cause clinical manifestations until weaning is started, neither increased rate of spontaneous abortion is known. In mice the absence of *Slc7a7* gene might cause an early severe metabolic derangement underlying the intrauterine growth failure.

**A metabolomics-guided screening of ENU mice for mouse models of human metabolic diseases.** *J. Wu<sup>1, 2</sup>, H. Kao<sup>1</sup>, S. Li<sup>1</sup>, R. Stevens<sup>3</sup>, S. Hillman<sup>3</sup>, D. Millington<sup>3</sup>, Y. Chen<sup>1, 3</sup>.* 1) Inst. of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Dept. Medical Research, China Medical College Hospital, Taichung, Taiwan; 3) Department of Pediatrics, Duke University Medical Center, Durham, North Carolina, USA.

Our laboratory has long interests in rapid analysis of metabolites at the whole-organism level (a metabolomics approach), using methods such as tandem mass spectrometry, HPLC and GC/MS. Here we reported the use of tandem mass spectrometry screening for amino acid and acyl carnitine profiles to detect the derangements in the pathways of amino acid and fatty acid metabolism in mice treated with the N-ethyl-N-nitrosourea (ENU). Treatment of ENU efficiently generated single-nucleotide mutation in mice. In a recessive screening of --- G3 mice from --- families, we identified mice with striking elevation of blood branched chain amino acids (BCAAs, valine, leucine/isoleucine 20-30 folds) and mice with elevation of hydroxy-butryl carnitine (C4-OH, 3 to 5 folds). Mice with elevation of C4-OH were clinically asymptomatic, however, mice with elevation of BCAAs were failure to thrive (body weight 60-70% of controls), weak with decreased spontaneous movement, hairs were thin and scanty and luster decreased, and many died before weaning. Urine organic acids showed the presence of -ketoisovaleric acid, -ketoisocaproic acid and -keto-methylvaleric acid; the branched chain ketoacids derived from valine, leucine and isoleucine, respectively. These clinical and laboratory findings resemble human maple syrup urine disease (MSUD) caused by the deficiency of branched-chain alpha-keto acid dehydrogenase (BCKD) complex. Affected mice responded to the MSUD diet low in the BCAAs, with amelioration of the clinical symptoms and normalization of the abnormal amino acid pattern. Our data suggested that the metabolomics-guided screening coupling with ENU mutagenesis is a powerful approach to uncover novel enzyme deficiency / important pathway and to generate mouse models for human genetic metabolic disorders.

**Biochemical and molecular basis of prognosis in nonketotic hyperglycinemia.** *J. Van Hove<sup>1, 4</sup>, V. Mahieu<sup>1</sup>, E. Schollen<sup>1</sup>, K. Vande Kerckhove<sup>1</sup>, J. Hennermann<sup>2</sup>, D. Applegarth<sup>3</sup>, J. Toone<sup>3</sup>, G. Mathijs<sup>1</sup>.* 1) Pediatrics, Catholic University Leuven, Leuven, Belgium; 2) Humboldt University Berlin, Berlin, Germany; 3) University of British Columbia, Vancouver, B.C., Canada; 4) University of Colorado Health Sciences Center, Denver, Colorado.

Nonketotic hyperglycinemia (NKH) is caused by deficient activity of the glycine cleavage system, consisting of the P-, T-, H-, and L- protein. In order to study prognostic determinants in NKH, 15 patients with NKH were categorized according to neurodevelopmental outcome. 8 patients with severe NKH made no developmental progress, required 2 to 4 anticonvulsants, and some had brain malformations. 4 patients with intermediate NKH made developmental progress up to DQ <25, and required 1 or no anticonvulsants. Mild NKH patients made developmental progress beyond DQ 25 following special education, and seizures were controlled with benzoate only. Mild NKH patients tended to have late-onset, and severe to intermediate NKH neonatal-onset, but several exceptions occurred. Low CSF/plasma glycine ratio (<0.08) only occurred in mild NKH. Benzoate therapy dosing was adjusted to achieve steady state low-normal plasma glycine levels; then the whole body glycine balance was calculated from the benzoate dose and the dietary glycine and serine intake, reflecting in vivo glycine cleavage activity. The whole body glycine balance differed between severe NKH (2 to 4.1 mmol/kg/day), and intermediate or mild NKH (< 2 mmol/kg/day). Residual glycine cleavage enzyme activity in vitro did not differ between prognostic categories. 6 patients had T-protein mutations with 11/12 alleles identified. A P-protein mutation was identified in 6 families with 10/12 alleles identified. In 3 patients with severe neonatal NKH, no mutation was identified despite sequencing all exons of the P, T, and H protein genes. The private nature of most identified mutations did not allow relating specific mutations to outcome.

Program Nr: 1607 from 2003 ASHG Annual Meeting

**Overexpression of human alanine:glyoxylate aminotransferase in E. coli.** *M. Coulter-Mackie, Q. Lian.* Dept Pediatrics, Univ British Columbia, Vancouver, BC, Canada.

Primary hyperoxaluria type 1, PH1, results from a deficiency of alanine:glyoxylate aminotransferase (AGT) activity. AGT is a liver peroxisomal enzyme in humans. The AGT gene occurs as either of two normal variants the major and minor alleles, the latter with an allele frequency of about 20% in the general population. We have inserted the cDNAs for both variants into pET vectors where the expression is under the control of the T7 promoter and can be induced with IPTG. E. coli expression is a suitable approach because AGT does not undergo post-translational modification. E. coli does not produce AGT endogenously although it does synthesize the AGT co-factor, PLP. The overexpression of AGT reaches levels of 20-25% of cellular protein. The AGT produced is enzymatically active. The cloned gene has been used to demonstrate the effects of missense PH1 mutations on activity. Normal and mutant AGT products have been compared with respect to their relative affinities for PLP. Normal AGT produced in E. coli has been used as a source of antigen for AGT-specific antibody production. A number of missense mutations have now been identified in the AGT gene and several have now been demonstrated to act synergistically with the polymorphic changes of the minor allele. Expression in E. coli provides a system where mutations, singly and in combinations can be generated easily, manipulations can be performed rapidly and the enzyme can be studied in isolation from effects of organelle transport chaperones. This work was funded by a grant from the Garrod Association of Canada and the Hospital for Sick Children Foundation.

**Functional characterization of a second mouse mitochondrial ornithine transporter, MmORNT2.** *J. Camacho, N.R. Camacho, D. Andrade.* Dept. Human Genetics, Univ Oklahoma , Oklahoma City, OK.

The Hyperornithinemia-Hyperammonemia-Homocitrullinuria (HHH) syndrome is a disorder of the urea cycle caused by a deficiency in the human mitochondrial ornithine transporter (HsORNT1). We have recently reported the characterization of a second human mitochondrial ornithine transporter (HsORNT2). HsORNT2 (5q31) is a functional retroposon with 88% identity to HsORNT1 (13q14) that is localized between two clusters of beta and gamma protocadherin genes and has the ability to rescue the deficient ornithine transport in cultured fibroblasts of HHH patients. We identified the mouse ORNT2 (MmORNT2) by screening the Expressed Sequence Tag and High Throughput Genome Scan (HTGS) databases. The MmORNT2 gene is 81% identical to HsORNT2, but has a single base deletion (AC-) in the predicted fourth codon. MmORNT2 (Ch18) is also intronless and localized between the beta and gamma protocadherin gene clusters. Northern and PCR analysis demonstrated MmORNT2 is expressed only in the testis. Functional studies demonstrated that wild type MmORNT2 did not rescue the deficient ornithine metabolism in cultured HHH fibroblasts. Immunofluorescence and in-vitro transcription/translational studies with a C-myc-tagged construct, demonstrated that MmORNT2 is not translated. We generated an N-myc-tagged-MmORNT2-Asn4 (Asn4 is conserved in several ORNT from different species) construct that targeted to the mitochondria but did not rescue the deficient ornithine transport of HHH fibroblasts. Examination of the *Saccharomyces*, *Neurospora*, *Drosophila*, *Gallus* and *C. elegans* HTGS databases did not reveal the existence of ORNT derived retroposons, but in the *Rattus* an intronless clone 78% identical to the rat ORNT1 was observed. Our results suggest that ORNT was duplicated a second time after the diversification of the Eutheria mammals into the rodent and New World Monkey. Most likely MmORNT2 lost its ornithine transporting capacity before the single base deletion occurred in the predicted fourth codon. Interestingly, MmORNT2 was copied from an autosomal MmORNT1 (Ch8) contrary to other testis specific retroposons that are duplicated from X-linked genes (i.e. Glycerol kinase).

**Functional and structural bases of carnitine palmitoyltransferase 1A deficiency.** *S. Gobin*<sup>1, 2</sup>, *L. Thuillier*<sup>1, 2</sup>, *G. Jogl*<sup>3</sup>, *A. Faye*<sup>1</sup>, *L. Tong*<sup>3</sup>, *A. Munnich*<sup>2</sup>, *J.P. Bonnefont*<sup>2</sup>, *J. Girard*<sup>1</sup>, *C. Prip-Buus*<sup>1</sup>. 1) INSERM U567, 24 Rue du Fg Saint-Jacques, Paris, France; 2) INSERM U393, 149 Rue de Sevres, Paris, France; 3) Department of Biological Sciences, Columbia University, New-York, USA.

Carnitine palmitoyltransferase 1A (CPT1A) mediates the transfer of long-chain fatty acids (LCFA) into liver mitochondria. Its tight inhibition by malonyl-CoA, the first intermediate of fatty acid biosynthesis, provides a physiological regulation of LCFA beta-oxidation and cellular fuel sensing. Human CPT1A deficiency is mostly characterized by hypoketotic hypoglycemia leading to coma with risk of sudden death or neurological sequelae. We have identified six CPT1A mutations, five of them being missense mutations (A275T, A414V, Y498C, G709E, and G710E) in a series of three CPT1A deficient patients. Their heterologous expression in *S. cerevisiae* allowed to validate them as disease-causing mutations. To gain further insights into their deleterious effects, we localized these mutated residues into a 3-D structure model of the human CPT1A that was created from the crystal structure of the mouse carnitine acetyltransferase (Jogl and Tong, 2003, Cell 112, 113). These combined functional and structural analyses demonstrated for the first time that disease-causing CPT1A mutations can be divided into two categories depending on whether they affect indirectly (structural determinant) or directly the active site of the enzyme (functional determinant). A275T, A414V and Y498C, which cause decreased catalytic efficiency, clearly belong to the first class. They are located at more than 20Å... away from the active site and mostly alter the stability of the protein itself and/or of the enzyme-substrate complex. By contrast, G709E and G710E mutations, which abolish CPT1A activity, belong to the second category. Indeed, they affect Gly residues that are not only essential for the structure of the hydrophobic core in the catalytic site, but also contribute to chain-length specificity of CPT isoforms. This study could provide basis for pharmacological therapy of CPT1A deficiency due to class I mutations by drugs, such as fibrates, known to upregulate expression of LCFA oxidation genes.

**Long-term follow-up of well treated nephropathic cystinosis patients.** *I. Bernardini, M. Ueda, C. Phornphutkul, D. Krasnewich, R. Kleta, W.A. Gahl.* Section on Human Biochemical Genetics, MGB, NHGRI, NIH, Bethesda, MD.

Cystinosis is an autosomal recessive inborn error of metabolism with intralysosomal cystine accumulation when untreated leads to glomerular kidney failure within the first decade of life and other nonrenal complications. The gene CTNS, mutated in cystinosis, codes for the protein cystinosin, which contains 367 amino acids and transports cystine out of lysosomes. A free thiol drug, cysteamine, depletes lysosomes of cystine, retards glomerular deterioration, and enhances somatic growth if begun in the first 2 years of life. Cysteamine is given orally every 6 hours at doses of 1.3-1.95 g/m<sup>2</sup> of free base or 60-90 mg/kg per day. Here we report the clinical outcomes of two siblings treated with cysteamine from the ages of 20 months (Pt. #1) and 2 weeks (Pt. #2). The siblings were compound heterozygous for the common 57 kb deletion involving CTNS and for a missense mutation in exon 12, c1015G>A (1354G>A), G339R. Both siblings complied well with oral cysteamine therapy. The leucocyte cystine value in Pt. #1 was 10.3 nmol half-cystine/mg protein (normal, < 0.2) at diagnosis; under treatment with cysteamine (35 mg/kg day at age 14 years), it was 0.5. Pt. #2 had a leucocyte cystine of 9.7 nmol half-cystine/mg protein at diagnosis and 0.3 on cysteamine (60 mg/kg day at age 8 years). Leucocyte cystines, checked yearly have reflected compliance with cysteamine therapy. Neither child received growth hormone. Pt. #1 is now 159 cm (10-25th percentile); Pt. #2 is 139 cm (90th percentile). The glomerular filtration rates, based upon 24 hour urine collections, are 84 and 101 ml/min 1.73m<sup>2</sup>, respectively. The patients' urines show signs of renal Fanconi syndrome, but both children have normal thyroid function. These cases illustrate the excellent clinical outcome possible for nephropathic cystinosis patients treated early and diligently. Early diagnosis is critical.

**Genetic and Biochemical dissection of a complex neurological disease: Leighs syndrome.** *J.M. Cameron, V. Levandovskiy, N. MacKay, B.H. Robinson.* Dept of Metabolism, Hospital for Sick Children, Toronto, Ontario, Canada.

Leighs disease is an early onset disease characterized by severe, progressive encephalopathy. Diagnosis of the disease is made by recognition of the distinct neurological symptoms and disease progression, but the actual cause of disease in a patient is variable: it can be associated with the deficiency of a number of enzymes involved in energy metabolism as well as with some mitochondrial DNA mutations. There are however, patients for whom no specific enzymatic deficiency can be attributed, representing half the patients in the literature.

We are conducting an exhaustive metabolic analysis of patients to determine the specific enzyme deficiencies causing disease (n=24). All known causes of Leigh's disease have been eliminated to select patients affected by novel mechanisms. We found that seven skin fibroblast cell lines displayed rates of 2-<sup>14</sup>C pyruvate to <sup>14</sup>CO<sub>2</sub> oxidation which were in the deficient range, despite the absence of any abnormality of PDH or the respiratory chain complexes: 0.600.05 (n=16) compared with control values of 1.530.18 (n=6). The rest of the Leighs patient group had oxidation rates of 1.700.18 (n=7), indistinguishable from controls.

These seven cell lines then underwent a further set of tests to evaluate capacity for ATP synthesis. This yielded one cell line with a major block in ATP synthesis with all substrates tested. Patient values (n=4) are shown for each substrate with average control values (four different control cell lines, each assayed up to four times): 10.783.08 [control=16.904.39 (no substrate)], 28.775.37 [control=108.3135.93 (5mM pyruvate, 1mM L-malate)], 27.687.05 [control=100.0130.35 (1mM L-malate, 5mM glutamate)], 38.6810.29 [control=84.1717.70 (1mM rotenone, 10mM succinate)] and 16.472.63 [control=26.925.64 (2mM antimycin, 2mM ascorbate, 0.1mM TMPD)].

Analysis of the defective cell lines for intracellular citrate and coenzyme A revealed further differences from control values. These findings are now being used to pinpoint the site of oxidative compromise and thus the identity of gene(s) responsible.

**Presence of Lactic Acidemia and Elevated Lactate-Pyruvate Ratio in Patients with Autism.** *S. Carlo*<sup>1,2</sup>, *N.J. Arciniegas*<sup>2</sup>, *D. Valencia*<sup>2</sup>, *J.R. Acevedo*<sup>3</sup>, *I. Ramos*<sup>2</sup>, *A.A. Reis*<sup>1</sup>, *J.M. Massanet*<sup>1</sup>, *N. Ramirez*<sup>2</sup>, *A.S. Cornier*<sup>1,2</sup>. 1) Dept Biochemistry, Ponce Sch Medicine, Ponce, PR; 2) Genetic Diagnostic Group, Mayaguez, PR; 3) University of PR, Arecibo, PR.

Autism is defined behaviorally as a syndrome consisting of abnormal social skills development, sensorimotor deficits, and verbal and non-verbal communication problems. The study of this disorder has intensified in the last few years because incidence has increased to 7 of every 10,000 children. Even though the etiology of autism is unknown, neuro-psychiatric problems, fragile X, lead poisoning, chromosomal disorders and metabolic diseases has been implied in the pathophysiology of the disease. The clinical phenotype of the disorder is wide and is divided according to the level of functioning of the individual ranging from autism as the most severe and Asperger syndrome being the least affected phenotype. During the years of 2001 and 2002, 65 patients with the diagnosis of autism or pervasive developmental disorder were evaluated at our clinics. Thorough past medical history and physical examination was performed, familygrams were gathered. Metabolic work up included plasma amino acid, lactate, pyruvate and ammonia levels, urine organic acids and if dysmorphic features were identified blood chromosomes were also included in the laboratory work up. Patients with chromosomal abnormalities were discarded from the study. Out of the 65 patients evaluated 3 were found with chromosomal abnormalities, and 49 returned with completed metabolic work up. In this group of 49 patients 18 had a diagnosis of autism and 31 of PDD, only one patient was female, average age was 5.4 years. Sixty percent of the patients had elevated lactate levels (normal levels 3 to 12 mg/dl); 37% had decreased pyruvate levels (normal levels 0.3 to 0.7 mg/dl) and 60% had elevated lactate/pyruvate ratio (normal 10 to 30). Even though these results are not conclusive, the fact that a high percentage of these patients presented lactic acidemia and elevated lactate/pyruvate ratio point toward the possibility of an oxidative metabolism disorder or an intra-mitochondrial disorder to be involved in the expression of the autism phenotype.

**Clinical and Molecular Genetics of ARC syndrome.** *P. Gissen*<sup>1, 2</sup>, *C.A. Johnson*<sup>1, 2</sup>, *K.M. Eastham*<sup>5</sup>, *P. McClean*<sup>3</sup>, *A.A.M. Morris*<sup>4</sup>, *J.E. Wraith*<sup>4</sup>, *S.A. Lynch*<sup>5</sup>, *O.W.J. Quarrell*<sup>6</sup>, *D.A. Kelly*<sup>2</sup>, *P.J. McKiernan*<sup>2</sup>, *E.R. Maher*<sup>1</sup>. 1) Section of Medical and Molecular Genetics, University of Birmingham, Birmingham, UK; 2) The Liver Unit, Birmingham Children's Hospital, Birmingham, UK; 3) St James's University Hospital, Leeds, UK; 4) Willink Biochemical Genetics Unit, Manchester, UK; 5) Department of Human Genetics, Newcastle Upon Tyne, UK; 6) Department of Medical Genetics, Langhill, Sheffield, UK.

ARC (arthrogryposis, renal dysfunction, cholestasis) syndrome (OMIM 208085), also known as Nezelof syndrome is a rare autosomal recessive metabolic disease. Fewer than 40 cases have been described in the literature. We have undertaken a UK national study of ARC to define the phenotypic expression and prognosis and provide a basis for gene mapping and identification. 8 ARC kindreds containing 21 patients were identified within the UK over the last 10 years. All cases were of Pakistani origin and all were born to consanguineous parents. The clinical features were reviewed: Liver pathology: All patients had a degree of cholestasis with low gamma glutamyl transpeptidase activity. However, the bilirubin level and elevation in liver transaminases vary from mild to moderate. Renal pathology: Most patients showed abnormalities on renal ultrasound examination. The typical clinical picture is of generalised aminoaciduria, associated with metabolic acidosis and glycosuria. These manifestations may appear at a later stage in the disease and are associated with intercurrent infections. Patients tend to develop nephrogenic diabetes insipidus with a variable time of onset. Arthrogryposis: All patients had hypotonia and severe motor delay. In the one family with 7 affected individuals 2 had no significant arthrogryposis while the other 5 had severe contractures of lower and upper limbs. Other manifestations: Platelet abnormalities were present in all investigated patients. 2 out of 21 patients had intracranial abnormalities. All patients failed to gain weight and suffered recurrent systemic infections. All patients died in the first year of life during systemic illness. Gene mapping studies are in progress. To date we have excluded linkage to ABCB11 ATP8B1.

**Natural history of isovaleric acidemia.** *R. Ensenauer<sup>1</sup>, S. Gruenert<sup>3</sup>, J. Willard<sup>1</sup>, D. Matern<sup>2</sup>, U. Wendel<sup>4</sup>, W. Lehnert<sup>3</sup>, K.O. Schwab<sup>3</sup>, M. Brandis<sup>3</sup>, A-W. Mohsen<sup>1</sup>, J. Vockley<sup>1</sup>.* 1) Dept Medical Genetics, Mayo Clinic, Rochester, MN; 2) Dept Laboratory Medicine & Pathology, Mayo Clinic, Rochester, MN; 3) University Children's Hospital Freiburg, Germany; 4) University Children's Hospital Duesseldorf, Germany.

**Background:** Isovaleric acidemia (IVA) is caused by a deficiency of isovaleryl-CoA dehydrogenase (IVD), an enzyme in leucine catabolism. Clinical presentation of IVA is extremely variable. The mechanism for this is unclear. We present the results of a study designed to characterize the clinical history of IVA and correlate it to molecular and biochemical phenotype. **Methods:** 22 patients with IVA diagnosed in Germany in the past 30 years were characterized clinically, biochemically (metabolite/enzyme analyses) and by molecular methods. **Results:** 27% of patients were detected by newborn screening, and 50% of patients were diagnosed in the first weeks of life, while the remainder presented in childhood. Most common signs of metabolic crisis were recurrent emesis and acidosis (70% and 55% of decompensations, respectively). No crises were observed after 11 years of age. Most patients showed normal growth (77%) and development (73%). The IQ did not correlate with the number of crises but was decreased with later diagnosis. Patients on carnitine had a similar number of crises per year of treatment to patients treated with carnitine and glycine. Differences in urine isovalerylglycine (IVG) and plasma C5 carnitine among patients did not correlate with clinical condition. Total metabolite excretion was the same on therapy with carnitine plus/minus glycine. IVD activity was undetectable in transformed lymphocytes from all patients. Immunoreactive enzyme protein in the lymphocytes correlated with clinical severity. 20 new *IVD* gene mutations were identified, and some correlation of genotype with phenotype was apparent by molecular modeling. **Conclusions:** Favorable neurological long-term outcome of patients with IVA depends on early diagnosis. Expanded newborn screening helps to prevent disease manifestation and allows early management. The data provide a basis for prospective therapeutic studies.

**Functional characterization of mutant -galactosidase A, identified from Korean patients with Fabry disease.** S-S. Kim<sup>1</sup>, Y. Kim<sup>1</sup>, G-H. Kim<sup>1</sup>, H-W. Yoo<sup>2</sup>. 1) Genomic Res Ctr for Gen Dis, Asan Medical Ctr, Seoul, Korea; 2) Dept of Ped, Asan Medical Ctr, Ulsan University College of Medicine, Seoul, Korea.

Fabry disease is an X-linked recessively inherited metabolic disorder, which results from the deficient activity of the lysosomal hydrolase alpha-galactosidase A (-Gal A) leading to the systemic deposition of glycosphingolipids with terminal alpha-galactosyl moieties. In previous study, we have identified mutations of -Gal A in Korean patients with Fabry disease, which resulted in substitution of E66Q, R112C and D266N amino acid residues. In order to evaluate the effects of these mutations on the -Gal A activity, we constructed E66Q, R112C, and D266N cDNA from a wild type -Gal A. COS-7 cells transfected with these mutant (R112C, D266N) cDNA showed complete loss of its enzyme activities (about 4 and 2 % of wild type -Gal A, respectively), except E66Q. In E66Q mutant form of -Gal A, it has a residual activity (about 30%), and then a optimal pH range of E66Q mutant form was between 4 and 5, same as the range of wild type. In *in vitro* stabilization study, the E66Q mutant enzyme was relatively unstable compared to wild type. Furthermore, those cotransfected with a mutant and a wild type cDNA showed a lower -Gal A activity than those with wild type alone (~50% of wild type alone), which suggested the dominant negative effect of this mutant.

**Respiratory chain complex III deficiency presenting as isolated cardiomyopathy.** *A. Maltret<sup>1</sup>, E. Vilain<sup>2</sup>, S. Lebon<sup>1</sup>, D. Genevieve<sup>1</sup>, S. Romano<sup>1</sup>, D. Sidi<sup>2</sup>, D. Bonnet<sup>2</sup>, A. Munnich<sup>1</sup>, A. Rotig<sup>3</sup>, P. Rustin<sup>3</sup>.* 1) Department de Genetique, Hopital Necker-Enfants Malades, Paris, France; 2) Department of Pediatrics, Hopital Necker-Enfants Malades, Paris, France; 3) INSERM U393, Hopital Necker-Enfants Malades, Paris, France.

Mitochondrial respiratory chain deficiency due to Ubiquinol cytochrome c reductase (CIII) deficiency is a rare and clinically heterogeneous condition. It represents between 5% to 10% of cases of respiratory chain deficiency. We reviewed the reported cases of CIII deficiency with known molecular bases and found that 13/27 reported cases had mitochondrial cardiomyopathy including 6 hypertrophic cardiomyopathies, 4 dilated cardiomyopathies, 1 ischemic cardiomyopathy and 2 Wolf-Parkinson-White syndrome. Most cases presented with neurological features and progressive exercise lactic acidosis and sometimes myoglobinuria. Moreover, 9/27 reported cases also displayed exercise intolerance. Here, we report on a 10-years-old boy with no familial history who had syncopes ascribed to a documented ventricular fibrillation. He was resuscitated by external cardioversion. After recovering, neurological examination was normal. The patient received an implantable cardiovector defibrillator (ICD) and is doing well after a 5 month follow-up. Heart ultrasound displayed asymmetric cardiac hypertrophy with no obstruction. Extensive blood and urinary metabolic screening was negative. Histopathological analysis of the endomyocardial biopsy was normal but investigation of respiratory chain activity in endomyocardial biopsy and skin fibroblasts displayed a decreased absolute CIII activity with an abnormal activity ratio with respect to other respiratory chain complexes suggestive of a CIII deficiency. Cytochrome b mutations were excluded by molecular studies. This observation and the review of the literature suggest that cardiomyopathy is frequent in CIII deficiency and, apart from exercise intolerance presentation, could be the presenting symptom of the disease.

**Abnormal localization of OCTN2 carnitine transporters in primary carnitine deficiency.** *N. Longo, N. Miller, C. Amat di San Filippo.* Dept Pediatrics and Pathology, Div Med Genetics, Univ Utah, Salt Lake City, UT.

Primary carnitine deficiency (OMIM 212140) is an autosomal recessive disorder of fatty acid oxidation characterized by hypoketotic hypoglycemia, encephalopathy, and acute liver failure in younger patients or by skeletal/cardiac myopathy in older children. It is caused by mutations in the OCTN2 transporter (encoded by the SLC22A5 gene) that is responsible for tissue carnitine uptake and renal reabsorption of filtered carnitine. Nonsense and missense mutations in this transporter have been identified in patients with primary carnitine deficiency. When expressed in CHO cells, some of the missense mutations completely abolished carnitine transport, while others retained residual carnitine transport activity. To determine the mechanism by which natural mutations impaired carnitine transport, normal and mutant OCTN2 transporters were tagged with the green fluorescent protein and their subcellular distribution was assessed using confocal microscopy and markers of intracellular organelles. The normal OCTN2 transporter localized on the plasma membrane and there was no overlap with markers of the endoplasmic reticulum or the Golgi. Several OCTN2 carnitine transporters carrying missense mutations, including R83L, R169W, G242V, W283C, Y447C, did not reach the plasma membrane. These transporters co-localized with markers of the endoplasmic reticulum, with an increased perinuclear distribution. Most of these mutations completely abolished carnitine transport in the patients fibroblasts and when expressed in CHO cells. By contrast, the R19P-, A142S-, A301D-, E452K-, Y449D-mutant OCTN2 transporters reached the plasma membrane in a manner indistinguishable from the wild type OCTN2 and retained residual carnitine transport activity. These results indicate that natural mutations in the OCTN2 carnitine transporter can impair carnitine transport by affecting maturation of the protein to the plasma membrane. Mutant OCTN2 transporters capable of reaching the plasma membrane have minimal residual carnitine transport activity, while mutants retained inside the cells are completely ineffective.

**NOVEL MOLECULAR DEFECT OF CARNITINE PALMITYL TRANSFERASE I DEFICIENCY (CPT I) IN SAUDI ARABIA.** *M.S. Rashid<sup>1</sup>, L. Ijlst<sup>2</sup>, J. Ruiten<sup>2</sup>, R.J. Wanders<sup>2</sup>, A.I. Al Aqeel<sup>1, 3</sup>.* 1) Dep. of Genetics, King Faisal Specialist Hospital & Research centre, Riyadh, Saudi Arabia; 2) Dep. of Clinical Chemistry, University of Amstradam, The Netherland; 3) Dep. Of Pediatrics, Riyadh Military Hospital, Riyadh, Saudi Arabia.

Carnitine Palmitoyl 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  $\beta$ -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 1574 insertion G in the first family which was confirmed at genomic level and 1950GA transition resulting in glycine 650 aspartic acid change (G650D) in the protein in the second family. 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 with unique molecular defect in Saudi Arabia, which may be treated easily with very good results if detected early in life.

**Identification of novel mutations in the human ornithine transcarbamylase(OTC) gene and transient expression of the mutant protein *in vitro*.** H-W. Yoo<sup>1</sup>, S-S. Kim<sup>2</sup>, G-H. Kim<sup>1</sup>. 1) Dept Pediatrics, Medical Genetics Clinic & Laboratory, Asan Medical Center, Ulsan University College of Medicine, Seoul, Korea; 2) Asan Medical Center, Genome Research Center for Birth Defects and Genetic Diseases, Seoul, Korea.

The urea cycle, consisting of a series of six enzymatic reactions, plays key roles to prevent the accumulation of toxic nitrogenous compound and synthesize arginine *de novo*. Ornithine transcarbamylase(OTC) deficiency is one of the most common inborn error of urea cycle, which is inherited in X-linked manner. This study was undertaken to characterize molecular defects in Korean patients with OTC deficiency and to correlate them with phenotypes. To investigate molecular lesions in the OTC gene of 22 unrelated Korean patients with OTC deficiency, coding regions with each intron-exon boundary were analyzed by PCR-direct DNA sequencing. Mutant OTC cDNAs were constructed by site-directed mutagenesis and cloned into pcDNA expression vector. After transient expression in COS-7 cells, the OTC activity was determined by HPLC method and Western blot was concomitantly performed using a monoclonal OTC antibody. We characterized molecular lesions in all patients examined. We identified 20 different mutations in 22 unrelated Korean patients; L9X, R26P, R26X, T44I, R92X, G100R, R141Q, N161S, G195R, M205T, M206R, H214Y, K221N, D249G, R277W, F281S, 853 delC, R320X, V323M and 796-805del. Among these, 10 mutations(L9X, R26P, R26X, G100R, K221N, D249G, F281S, V323M, 853 delC, and 796-805del) were novel. The L9X, R26P, and R26X are presumed to lead to the disruption of leader sequences, required for directing mitochondrial localization of the OTC precursor. Their phenotypes were severe, presenting at the neonatal onset. The G100R, R277W, and V323M mutations were uniquely identified in patients presenting at the late onset, with the residual enzyme activity *in vitro* expression. Five mutations(T44I, N161S, G195R, M206R, and R320X) were found in female heterozygote patients without the residual enzyme activity *in vitro* expression. The other genotypes were associated with the phenotype presenting at the neonatal onset.

**Prolidase deficiency in four Amish patients: a novel mutation.** *H. Wang*<sup>1,2</sup>, *B.T. Kurien*<sup>4</sup>, *D. Lundgren*<sup>3</sup>, *N.C. Patel*<sup>4</sup>, *K.M. Kaufman*<sup>4</sup>, *D.L. Miller*<sup>4</sup>, *J. Tumbush*<sup>1</sup>, *D.S. Kerr*<sup>2</sup>, *S. Kurono*<sup>5</sup>, *H. Matsumoto*<sup>5</sup>, *R.H. Scofield*<sup>4</sup>. 1) DDC Clinic Special Needs Child, Middlefield, OH; 2) Dept of Pediatrics, Rainbow Babies & Childrens Hosp, Case Western Reserve University, Cleveland, OH; 3) Biochemical Genetics Laboratory, MetroHealth Medical Center, Cleveland, OH; 4) Oklahoma Medical Research Foundation, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 5) University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Prolidase is a cytosolic enzyme consisting of 493 amino acids that catalyzes hydrolysis of dipeptides with a C-terminal proline or hydroxyproline. Prolidase deficiency (PD) is a rare autosomal recessive disease with less than 50 cases reported in the literature. Recently, a total of four Amish patients were diagnosed with prolidase deficiency in the Geauga Amish settlements of Ohio. A fifth patient, a sibling of one of the four, had a typical clinical course of prolidase deficiency but died from infection in 1998 before confirmation of PD. The five patients were from three families with varying degrees of consanguinity. Two of the three marital-unions were between second cousins and the third were fourth cousins. The common ascendants can be traced back to the seventh generation. The typical presentations of the disease are recurrent infections, petechiae, thrombocytopenia and hepatosplenomegaly. They all have characteristic facies including prominent forehead, ocular hypertelorism and proptosis. The classic skin ulcers developed on lower extremities in three of the five patients. High amounts of iminodipeptides were detected in urine of all diagnosed patients but not in any of the 11 direct family members tested. Serum prolidase activity was measured by mass spectrometry. All patients had undetectable serum prolidase activity. Direct sequencing of PCR amplified genomic DNA from the three homozygous patients revealed the same single nucleotide polymorphism (C-to-T in Exon 11) in each. This resulted in an arginine being replaced by a premature stop-codon at amino acid residue 265. This novel mutation is thought to have resulted in the production of a non-functional polypeptide, which is consistent with both the laboratory and clinical findings.

**Disruption of the 3--hydroxysterol 24-reductase Gene: Development and Characterization of a Murine Model of Desmosterolosis.** C.A. Wassif, F.D. Porter. HBD, NICHD/NIH, Bethesda, MD.

Desmosterolosis is a rare multiple malformation/mental retardation syndrome due to an inborn error of cholesterol synthesis. Desmosterol is a sterol that is normally found in the developing nervous system. Reduction of desmosterol by the 3--hydroxysterol 24-reductase produces cholesterol. Desmosterolosis is due to mutation of the 3--hydroxysterol 24-reductase gene (*DHCR24*). In order to further our understanding of the pathophysiological processes which underlie this human malformation syndrome and the more common Smith-Lemli-Opitz syndrome, we produced a mouse model of desmosterolosis by disrupting the *Dhcr24* gene in mouse embryonic stem cells. *Dhcr24* +/- mice were phenotypically normal, thus they were intercrossed to obtain homozygous mutant pups. *Dhcr24* -/- pups were not viable. The mutant embryos are however alive just prior to birth at embryonic day 18.5 (E18.5). Sterol analysis of serum, liver, kidney, cortex and midbrain from E18.5 *Dhcr24* -/- embryos showed significantly increased levels of desmosterol compared to tissues from either *Dhcr24* +/+ or *Dhcr24* +/- embryos. Both cholesterol and total sterol levels were significantly decreased in mutant embryonic tissues. Phenotypic examination and detailed histological analysis revealed no significant differences between *Dhcr24* -/- and control E18.5 embryos. This later result is in distinct contrast to teratogenic models of desmosterolosis using triparanol and is in contrast to our previously reported SLOS and lathosterolosis mouse models. It is likely that the differences observed in these three mouse models are due to differences in the respective ability of desmosterol, 7-dehydrocholesterol and lathosterol to substitute for cholesterol during development. We suspect, and will investigate the possibility, that the neonatal death of the desmosterolosis mice is related to neuronal dysfunction.

**Making the diagnosis of alkaptonuria: Unusual skin findings in an alkaptonuria patient.** *C. Phornphutkul<sup>1</sup>, P. Suwannarat<sup>1</sup>, W. Introne<sup>1</sup>, I. Bernardini<sup>1</sup>, M. Turner<sup>2</sup>, R.D. Heekin<sup>3</sup>, R. Kleta<sup>1</sup>, W.A. Gahl<sup>1</sup>.* 1) Section on Human Biochemical Genetics, MGB, NHGRI, NIH, Bethesda, MD; 2) Dermatology Branch, NCI, Bethesda, MD; 3) Orthopaedic Surgery Service, Walter Reed Army Medical Center, Washington, DC.

Alkaptonuria is a rare inborn error of metabolism in which the degradation of tyrosine is compromised by decreased activity of homogentisate 1,2 dioxygenase leading to increased homogentisic acid (HGA) in the body. Alkaptonuria is characterized by vertebral disk collapse, arthritis, joint destruction requiring replacement, and ochronosis, or dark pigmentation of tissues due to HGA and its oxidation products. The increased urinary excretion of HGA, and its subsequent oxidation, often causes blackening of the urine ("alkaptonuria") and leads to the diagnosis in early childhood. In 58 patients seen at the National Institutes of Health Clinical Center, the diagnosis of alkaptonuria was made based upon dark urine in 32. In the remaining 26 patients, the diagnosis was suspected by finding black cartilage upon surgical replacement of joints or ochronotic ears or eyes in adulthood. Here we report unusual skin findings in a 46 year old Florida fisherman who never had darkening of his urine. The patient presented with clearly visible blackening of his nose, first noticed 4 years previously, and of his hands (lateral aspects of thumb and index finger), first noticed 7 years prior to our examination. He also showed typical ochronotic pigmentation of his ears, noticed 10 years previously. The patient underwent left arthroscopic knee surgery because of increasing pain. Black cartilage and black menisci prompted the orthopedic surgeon to consider alkaptonuria. An elevated urinary excretion of 4000 mg/day of HGA (normal, < 40) confirmed the diagnosis of alkaptonuria. The patient had no renal insufficiency, which could exacerbate the ochronosis of the skin and cartilage. Blackening of sun-exposed skin areas besides the ears and eyes should alert physicians to the possible diagnosis of alkaptonuria.

**Effect of vitamin C on collagen and lysyl hydroxylase RNA in fibroblasts from patients with Ehlers-Danlos syndrome type VI.** *E. Schwarz*<sup>1</sup>, *N. Longo*<sup>2</sup>, *M. Pasquali*<sup>2</sup>. 1) ARUP Inst Clin Exp Pathol; 2) Dept Pathology and Pediatrics, University of Utah, Salt Lake City, UT.

Ehlers Danlos Syndrome type VI (EDS VI, OMIM #225400, kyphoscoliotic type) is a recessive disorder of connective tissue characterized by hyperextensible skin, joint hypermobility, scoliosis and ocular fragility. This disease is caused by defective lysyl hydroxylase (PLOD1), the vitamin C dependent enzyme that converts lysyl residues to hydroxylysine in procollagen peptides. Hydroxylysine is essential for the formation of pyridinium cross links, pyridinoline (Pyr) and deoxypyridinoline (Dpyr), in mature collagen. Pyr and Dpyr are excreted in the urine as products of collagen degradation, with a Dpyr/Pyr ratio = 0.20-0.30 in normal controls. Patients with EDS VI have an abnormally high Dpyr/Pyr ratio (>4), which is diagnostic for this disease. There are at least two additional lysyl hydroxylases (PLOD2 and PLOD3) with different substrate specificity that are encoded by genes not defective in EDS VI. Vitamin C improves the clinical manifestations of Ehlers-Danlos syndrome type VI. Here we evaluate the effect of vitamin C on collagen, pyridinium cross links production, and mRNA levels for different types of lysyl hydroxylase (PLOD1, PLOD2, and PLOD3). Vitamin C increased collagen production and pyridinium cross-link formation in long-term cultures of normal and EDS VI fibroblasts. However, in normal fibroblasts vitamin C decreased the relative abundance of Dpyr while it had the opposite effect in fibroblast of patients with EDS VI, reproducing the Dpyr/Pyr ratio observed in vivo. Vitamin C increased mRNA levels for PLOD1 and PLOD2, but did not affect significantly PLOD3 mRNA levels in normal cells. Some cells of patients with EDS VI had no detectable levels of PLOD1 mRNA and vitamin C could not restore normal levels. However, PLOD2 responded to vitamin C as in control cells. These results indicate that vitamin C might improve clinical symptoms in EDS VI by stimulating collagen production and cross link formation. This effect could be mediated, at least in part, by stimulating one form of lysyl hydroxylase (PLOD2) not impaired in EDS VI.

**Decreased abundance of epidermal growth factor receptor (EGFR) in galactose-1-phosphate uridylyltransferase (GALT) -deficient patient cells.** *T. Slepak, K. Lai.* The Dr. John T Macdonald Foundation Center for Medical Genetics, Department of Pediatrics, University of Miami, Miami, FL.

In humans, deficiency of GALT (E.C. 2.7.7.12) produces the disorder Classic Galactosemia. Although newborn screening and a galactose-restricted diet prevent the acute toxicity associated with this disease, secondary complications such as premature ovarian failure persist in some patients. The pathogenic mechanisms producing dysfunction of multiple organs remain largely unknown. Previously we demonstrated, in galactosemic patient fibroblast cell lines, reduced level of glycoproteins that are specifically detected by the *Sambucus nigra* agglutinin (SNA) (Lai et al. (2003), *Glycobiology* 13: 285-94). SNA lectin recognizes terminal sialic acid linked (2-6) to the penultimate galactose molecule of the oligosaccharide chains found in the complex glycoproteins. In this new study, we revealed that glycosylation defects in GALT deficiency are not be limited to the absence of (2-6)-linked sialic acids, but also terminal sialic acid linked (2-3) to the galactose molecules of the complex sialylated carbohydrate chains and (2-3)-linked sialic acids in o-glycans.

Aberrant protein glycosylation has been reported in galactosemic patients, but the few proteins involved were limited to secreted or cytosolic proteins. In all cases, aberrantly glycosylated proteins exhibited altered biological activities. Since many membrane proteins are glycoproteins, we decided to examine if aberrant glycosylation occurs in membrane proteins when GALT is deficient. In Western Blot analysis using anti-EGFR antibody, we compared the abundance of EGFR in two primary fibroblast cell lines derived from Classic Galactosemic patients and cell lines derived from normal controls. We found significantly reduced level of EGFR proteins in both galactosemic cell lines, but such decrease was not accompanied by the corresponding decrease in EGFR mRNA level. This study is significant as we demonstrated for the first time that normal galactose metabolism is needed for normal EGFR function and the galactose metabolic pathway can provide novel cancer therapeutic targets.

Program Nr: 1625 from 2003 ASHG Annual Meeting

**Clinical research center for neuronal ceroid lipofuscinoses (NCLs) at the New York State Institute for Basic Research in Developmental Disabilities.** *N. Zhong, W. Ju, A. Wronska, A. Golabek, E. Kida, S. Sklower-Brooks, M. Keogh, M. Rattazzi, J. Currie, M. Flory, R. Freedland, R. Kascsak, J. Vietze, C. Wang, P. Vietze, W.T. Brown, K.E. Wisniewski.* New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY10314.

Neuronal ceroid lipofuscinoses (NCLs) are a group of progressive neurodegenerative disorders characterized by intralysosomal accumulation of ceroid lipopigment. The NCLs consist of at least eight forms, for which six genes have been identified, and possibly more variants are being recognized. Although research studies are making progress for this group of lethal disorders, the nature of the clinical process and the progression of the NCLs are yet unclear. Therefore, a clinical research center has been formed recently at the New York State Institute for Basic Research in Developmental Disabilities (IBR) to accelerate clinical research studies for NCLs, with an emphasis on clinical diagnosis and treatment. Currently, we are maintaining the largest worldwide NCL database and NCL cell lines, having a broad connection with, and support of, national and international family groups and the international NCL clinical research consortium. We are performing clinical evaluations, including neuropathological studies, biochemical enzyme test, and DNA mutation analyses, for NCLs. A phase II clinical trial of Cystagon™ treatment on four patients with NCL1, the infantile onset of NCL, underlied by gene CLN1, is being studied. In addition, identification of uncharacterized NCL families, demonstrating new biomarkers, correlation of genotypes with phenotypes, determination of the epidemiology of NCLs, and searching for a novel therapeutic approach are the major focuses being carried out. For more information, please visit [www.NCLatIBR.com](http://www.NCLatIBR.com), or e-mail to [NCL.IBR@OMR.STATE.NY.US](mailto:NCL.IBR@OMR.STATE.NY.US).

**UDP-GlcNAc 2-epimerase activity in Hereditary Inclusion Body Myopathy.** *S. Sparks*<sup>1</sup>, *M. Lalor*<sup>1</sup>, *E. Orvisky*<sup>2</sup>, *M. Huizing*<sup>1</sup>, *D. Krasnewich*<sup>1</sup>, *M.-S. Sun*<sup>1</sup>, *M. Dalakas*<sup>3</sup>, *W. Gahl*<sup>1</sup>. 1) Medical Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) NSB, NIMH, NIH, Bethesda, MD; 3) NINDS, NIH, Bethesda, MD.

Uridine diphosphate-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (UDP-GlcNAc 2-epimerase/ManNAc kinase), encoded by the *GNE* gene on 9p12-13, is the bifunctional and rate-limiting enzyme involved in the biosynthesis of sialic acid (N-acetylneuraminic acid). The epimerase converts UDP-GlcNAc to N-acetylmannosamine (ManNAc) and the kinase converts ManNAc to ManNAc-6-phosphate. Sialic acid, as a terminal sugar on cell surface oligosaccharides, is important in cell-cell interactions and signal transduction. Mutations in either or both the epimerase and kinase domains of *GNE* cause Hereditary Inclusion Body Myopathy (HIBM), an autosomal recessive disorder characterized by adult-onset, progressive distal and proximal muscle weakness sparing the quadriceps muscle. We employed cultured fibroblasts as a model system to investigate epimerase activity in cells from three HIBM patients. Patient #1, with two epimerase domain mutations (G455T and C787T) had epimerase activity (measured by conversion of <sup>3</sup>H-UDP-GlcNAc to <sup>3</sup>H-ManNAc) 38% of control (n=2). Patient #2, with one epimerase mutation (T698C) and one kinase mutation (C1943T), had epimerase activity 48% of control (n=4). The third patient (#3), with homozygous kinase mutations (T2186C), had epimerase activity 83% of control (n=4). These findings indicate a rough correlation of epimerase activity with the number of *GNE* epimerase domain mutations.

**Fatal Medium-Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency in a previously a symptomatic 8-year old female.** *N. Quintanilla*<sup>1</sup>, *R. Garola*<sup>2</sup>, *G. Allen*<sup>3</sup>, *M. Dasouki*<sup>4</sup>. 1) Department of Pathology, Truman Medical Center, Kansas City, MO; 2) Department of Pathology, Children's Mercy Hospitals & Clinics, Kansas City, MO; 3) Sec Pediatric Intensive Care, Children's Mercy Hospitals & Clinics, Kansas City, MO; 4) Sec Med Genet & Molec Med, Children's Mercy Hospitals & Clinics, Kansas City, MO.

MCADD is the most common mitochondrial fatty acid oxidation defect, usually manifest during infancy with hypoglycemia, acute encephalopathy and occasionally with sudden, un-explained death. Newborn screening may prevent morbidity and mortality associated with MCADD. Herein we describe a previously healthy 8-year old girl with MCADD diagnosed on post-mortem examination. She was found unresponsive at home. The day before, she complained of stomachache, dizziness, headache and fever. On admission, she was febrile and unresponsive. EEG was consistent with subclinical status epilepticus; a head CT scan showed diffuse cerebral edema and features of severe hypoxic insult. She had leukocytosis with left shift; hyperglycemia, no ketones in urine, increased creatinine and BUN; elevated liver enzymes, prolonged PT and PTT and elevated CPK. Meningoencephalitis was suspected. Over the following three days, her hepatic and renal function deteriorated further and the neurologic exam was consistent with brain death. Post-mortem examination revealed marked jaundice, anasarca, submassive hepatic necrosis with macro and microvesicular steatosis. Lipid deposits were not found in myocardium or skeletal muscle. Brain showed diffuse ischemic hypoxic changes. Swollen mitochondria with fragmented cristae were seen on EM. No morphologic or microbiologic evidence of meningoencephalitis was found. Elevated plasma medium chain acylcarnitines were consistent with MCADD. DNA extracted from skin fibroblasts was homozygous for the "A985G" MCAD mutation. In addition to this patient, only 2 other previously asymptomatic older MCADD patients were reported in the literature. A 30-year old man with rhabdomyolysis [Ruitenbeek et al, J Neurol Neurosurg Psychiatry, 1995] and a 19-year old year woman with fatal encephalopathy [Yang et al, Mol Genet Metab, 2000].

**Mitochondrial porin deficient mice: a Murine model for Mendelian respiratory chain defects and encephalopathy.** *F. Scaglia<sup>1</sup>, Z.J. Cai<sup>1</sup>, K. Anflous<sup>1</sup>, D.A. Armstrong<sup>2</sup>, E. Weeber<sup>3</sup>, J.D. Sweatt<sup>3</sup>, W.J. Craigen<sup>1</sup>.* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept Pathology, Baylor Col Medicine, Houston, TX; 3) Div Neuroscience, Baylor Col Medicine, Houston, TX.

Voltage dependent anion channels (VDACs; porins) are small pore-forming proteins that form the main pathway for metabolites across the mitochondrial outer membrane. VDACs 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. To determine the physiological function of each isoform, gene targeting has been used to delete them in cultured cells and mice. VDAC1<sup>(-/-)</sup>, VDAC3<sup>(-/-)</sup>, and VDAC1/3<sup>(-/-)</sup> mice have been generated and exhibit abnormalities in spatial, and associative learning, and hippocampal synaptic plasticity that are reminiscent of the neurological deficits seen in patients with respiratory chain (RC) defects.

Since a reduction in cytochrome c oxidase activity is observed in the skeletal muscle of VDAC1<sup>(-/-)</sup> mice, we evaluated whether brain mitochondria from VDAC<sup>(-/-)</sup> mice exhibit any RC defect that could correlate with the observed cognitive deficits. Mitochondrial RC enzyme assays of brain mitochondria of VDAC deficient mice reveal significant RC defects. Immunoblotting and differential display exhibit reduced expression of brain RC proteins. Aconitase activity is reduced in the brain of VDAC1/3<sup>(-/-)</sup> mice. Preliminary data suggest that there is an increased oxidative modification of proteins by reactive oxygen species (ROS) in the brain of VDAC<sup>(-/-)</sup> mice. These data point to a role for VDACs in the modulation of the mitochondrial RC. Further experiments are being carried out to evaluate whether this regulation may be exerted via the flux of small metabolites across the mitochondrial outer membrane, altered calcium homeostasis, or through impaired mitochondrial translation.

**A WORLDWIDE COLLABORATION TO CAPTURE ALL HUMAN MUTATIONS.** *R. Cotton<sup>1</sup>, O. Horaitis<sup>1</sup>, A.D. Auerbach<sup>2</sup>, A.F. Brown<sup>3</sup>, S. Callaghan<sup>4</sup>, A.J. Cuticchia<sup>5</sup>, J.T. den Dunnen<sup>6</sup>, P.M. Nadkarni<sup>7</sup>, C.R. Scriver<sup>8</sup>, S. Teebi<sup>9</sup>, and a consortium of the Human Genome Variation Society.* 1) Genomic Disorders Res Ctr, Melbourne, and The University of Melbourne, Dept of Medicine, Melbourne 3010, Australia; 2) Laboratory of Human Genetics & Hematology, The Rockefeller University, New York, NY, U.S.A; 3) MRC Human Genetics Unit, Edinburgh EH4 2XU, UK; 4) Victorian Bioinformatics Consortium, Monash University 3800, Australia; 5) Research Triangle Institute, North Carolina, U.S.A; 6) Leiden University Medical Center, Leiden, The Netherlands; 7) Yale University, Connecticut, USA; 8) McGill University, Montreal, Canada; 9) Hospital for Sick Children, Toronto, Canada.

There has been an exponential rise in the number of disease causing mutations discovered in the last few years. To ensure future availability to health care professionals, researchers and others, it is essential that this information, together with the corresponding phenotype, is collected, quality controlled, documented and stored safely. So far there has been no systematic way to collect, store and display all of these data while general databases such as OMIM or HGMD only store selective parts. Through their interactions, a number of members of the Human Genome Variation Society have addressed many problems regarding these issues such as nomenclature, standard software to curate mutations in gene specific databases, a WayStation to collect and review new mutations from research and diagnostic laboratories and a Central database to store and display these mutations and their associated phenotypes. Some of these projects are well developed for example the WayStation will begin collecting mutations shortly, Editors for 391 genes have already been recruited and nomenclature is now well defined for the commonest types of mutation with work continuing on systematically naming the more complex types. Other projects such as dedicated specialized software for locus specific databases are in the early stages of development. The central database is at the planning stage whilst phenotype representation has been planned. A source of all this information is available at [www.hgvs.org](http://www.hgvs.org).

**Delineating the OI/EDS Region of the 1(I) Chain and the Shared Mechanism of OI/ED and EDS VIIA.** *W.A.*

*Cabral*<sup>1</sup>, *E. Makareeva*<sup>2</sup>, *S. Leikin*<sup>2</sup>, *A. Colige*<sup>3</sup>, *A.D. Letocha*<sup>1</sup>, *J. Ty*<sup>1</sup>, *H.N. Yeowell*<sup>4</sup>, *G. Pals*<sup>5</sup>, *J.C. Marini*<sup>1</sup>. 1) BEMB, NICHD, NIH, Bethesda, MD; 2) Section on Physical Biochemistry, NICHD, NIH, Bethesda, MD; 3) Laboratory of Connective Tissue Research, University of Liege, Liege, Belgium; 4) Division of Dermatology, Duke University Medical Center, Durham, NC; 5) Dept of Human Genetics, VU Medical Center, Amsterdam, The Netherlands.

Patients with OI/EDS are a distinct subset of osteogenesis imperfecta patients. In addition to OI skeletal fragility, they have characteristics of EDS, including severe joint laxity and scoliosis. In 7 children with OI/EDS, we delineated mutations in the first 90 residues of the helical region of the 1(I) chain. We have determined that these collagen mutations cause abnormal N-propeptide processing, incorporation of pN-collagen into matrix and decreased dermal fibril diameters.

Mutations were identified by sequencing of RT-PCR products of 1(I) mRNA, since the proband collagen was electrophoretically normal. On Differential Scanning Calorimetry, all mutants show decreased stability of collagen compared to procollagen, suggesting that the secondary structure of the mutant collagen is stabilized by the presence of the N-propeptide. In vitro cleavage with N-proteinase processed only 25% of proband pro1(I) chains for exon 7 mutations, and 65-85% of pro1(I) chains for exon 8-11 mutations. Pericellular processing of proband procollagen was also delayed. pN-1(I) collagen is incorporated into matrix deposited by cultured fibroblasts and is prominent in newly incorporated and immaturely cross-linked fractions. Electron microscopy of dermal biopsies from 6 probands revealed smaller fibrils than matched controls, as is seen in EDS VII.

The data delineate a folding region of the 1(I) chain in which mutations alter the secondary structure of the N-proteinase cleavage site, causing N-propeptide retention and limiting fibril diameter. Thus the mechanism of EDS symptoms in OI/EDS patients is shared with patients who have EDS VIIA due to absence of the N-proteinase cleavage site.

**Physical Performance Measure for Individuals with MPS I.** *S. Haley<sup>1</sup>, M.A. Fragala<sup>1,2</sup>, H.M. Dumas<sup>2</sup>, A. Skrinar<sup>3</sup>.*  
1) Rehabilitation Sci, Sargent Col Health & Rehab Sci, Boston, MA; 2) Franciscan Hospital for Children, Boston, MA;  
3) Genzyme Corporation, Cambridge, MA.

**Objective:** To develop a physical performance measure (PPM-MPS I) for individuals with Mucopolysaccharidosis I (MPS I), a lysosomal storage disorder that results in impaired joint mobility and cardiovascular endurance. **Methods:** Twelve motor performance and two functional endurance items were developed based on a comprehensive literature review, feasibility in various clinical settings, and equipment and training needs. A script was constructed for administration, and instructions were developed for rating each scale. Following trial administration, five expert clinicians provided feedback for face validity and script utility. The final PPM-MPS I performance items are as follows: Subtest I-Arm Function (7 items), Subtest II-Leg Function (5 items), and Subtest III-Functional Endurance/Walking Efficiency (2 items). Pilot data were collected for nine subjects (ages 6- 29 years) with MPS I. **Results:** Seven of nine subjects completed all Arm Function items but speed of performance varied. All subjects exhibited reduced shoulder mobility. Only two of nine subjects completed all five Leg Function items. Subjects had most difficulty with stand to squat. Comfortable and fast walking speeds were consistently below norms for subject ages. Energy expenditure values were lower with comfortable walking speeds than fast speeds. **Conclusions:** The PPM-MPS I is designed for administration by clinicians as a comprehensive motor performance assessment of common functional limitations. These data confirm that individuals with MPS I have difficulty with tasks using arms and legs and experience overall reductions in functional endurance. PPM-MPS I can be administered at pre-determined intervals before, during and following intervention to quantify changes in arm, leg and cardiovascular function, or to monitor the natural history of disease progression. Further work is needed to develop normative scores for rating performance and speed and for determining minimal clinically important differences of the PPM-MPS I for future intervention trials.

**Bone and Joint Disease in the Mucopolysaccharidoses.** *C. Simonaro*<sup>1</sup>, *M. D'Angelo*<sup>2</sup>, *M. Haskins*<sup>3</sup>, *E. Schuchman*<sup>1</sup>. 1) Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY; 2) Dept Anatomy, PCOM, Philadelphia, PA; 3) Dept Pathobiology, U Penn, Sch of Vet Med, Philadelphia, PA.

A major feature of the mucopolysaccharidoses (MPS) is abnormal cartilage and bone development leading to short stature, dysostosis multiplex, and degenerative joint disease. We have previously shown that articular chondrocytes from MPS animals undergo apoptosis due to glycosaminoglycan storage and stimulation by proinflammatory cytokines, including TNF-alpha and IL-1beta. However, despite the enhanced apoptosis, no extensive cell loss was observed. To investigate this further, we performed BrDU incorporation studies and found that MPS chondrocytes had increased proliferation rates. This was explained by elevated levels of TGF-beta in the MPS cartilage, a growth factor known to prevent bone degeneration by enhancing cell proliferation and counteracting the deleterious effects of IL-1beta. However, despite elevated TGF-beta expression and enhanced chondrocyte proliferation in the MPS animals, extensive bone and joint disease was observed, suggesting that other factors were involved in promoting the degenerative effect. Therefore, we investigated the expression of metalloproteinases (MMPs) in the MPS animals since proinflammatory cytokines, such as TNF-alpha and IL-1beta, have been shown to upregulate MMP gene expression. The activity and mRNA expression of two MMPs known to be involved in the pathogenesis of arthritis, MMP-2 and MMP-9, were elevated in the MPS animals when compared to age-matched, normal controls. Overall, these results suggest new therapeutic targets for the MPS disorders, including drugs that inhibit apoptosis, proinflammatory cytokines, and/or MMPs, or drugs that augment the protective effect of TGF-beta.

**Subtle neuropsychological abnormalities in patients with type I Gaucher disease.** *D. Elstein, J. Guedalia, P. Rosenberg, V. Antebi, Y. Arnon, A. Zimran.* Gaucher Clinic, Shaare Zedek Medical Ctr, Jerusalem, Israel.

Because of a complaint of memory loss in a patient participating in the clinical trial with miglustat, an oral substrate inhibitor for Gaucher disease, a battery of tests relating to frontal-parietal lobe function was administered to trial participants plus patients receiving enzyme replacement therapy or untreated. The purpose of this study was to tease out the concern about drug-related cognitive dysfunction among patients with type I Gaucher disease. The tests administered were: MiniMental State Examination, Rey Osterreith Complex Figure Test, Rey Auditory Verbal Learning Test, Word Fluency Test (FAS), Semantic Fluency Test (Animals), Trail Making Tests A and B, Tower of Hanoi, Wisconsin Card Sorting Test. Of 108 patients, 55 had received miglustat, 31 were enzyme-treated, and 22 were untreated. The z-scores for all tests for all groups were below normal except for the MiniMental test, which were normal. In the miglustat group, there was no correlation between individual scores and duration of therapy. Based on these results, the clinical trial, which had been halted, was re-instated; miglustat today is approved for marketing in Europe and Israel. Like other glycolipid storage disorders, Gaucher disease phenotypic expression is probably a continuum from virtually asymptomatic to lethal neurological involvement. This study underscores visual-spatial defects that correlate with parietal lobe dysfunction. Albeit subtle, the aberrant results are pervasive among all patients tested and imply that there is a nidus of neurological pathology even in patients with the mutation that is protective of neuronopathic involvement.

**Do mutations in the glucocerebrosidase gene modify the course of Parkinson disease? Family studies in patients with Gaucher disease.** *O. Goker-Alpan*<sup>1, 2</sup>, *R. Schiffmann*<sup>3</sup>, *A. Lwin*<sup>1, 2</sup>, *E. Sidransky*<sup>1, 2</sup>. 1) NSB/NIMH,NIH, Bethesda, MD; 2) MGB/NHGRI, NIH, Bethesda, MD; 3) DMNB/NINDS, NIH, Bethesda, MD.

Gaucher disease, the inherited deficiency of glucocerebrosidase, can present with a wide range of symptoms of varying severity affecting the skeletal, hematologic and nervous systems. Parkinsonian symptoms are now included in the spectrum of associated neurologic manifestations based upon the concurrence of Gaucher disease with parkinsonism in about 20 recently reported cases. Parkinson disease, the second most common neurodegenerative disorder, results from multiple etiologies. Genetic, epigenetic and environmental factors may all contribute to this phenotype. In an earlier report, we showed that patients with Gaucher disease and parkinsonism have an increased number of family members affected with Parkinson disease. In this study, we present six unrelated families of probands with Gaucher disease, in which eight obligate carriers of glucocerebrosidase gene mutations had signs and symptoms of parkinsonism. The parkinsonian symptoms were associated with an early age of onset, often with an atypical course. Three families carried the L444P allele; one a recombinant allele and one the N370S mutation. It has been suggested that mutant glucocerebrosidase may be a risk factor contributing to early onset, L-dopa refractory parkinsonian symptoms. These findings provide further evidence that glucocerebrosidase gene mutations, even in the heterozygous state, might act as modifiers, altering the phenotype in individuals genetically prone to Parkinson disease.

**Mucopolysaccharidosis type IIIB: Identification of the causative mutation in the canine model.** *N.M. Ellinwood<sup>1</sup>, P.S. Henthorn<sup>2</sup>, U. Giger<sup>2</sup>, M.E. Haskins<sup>1</sup>*. 1) Dept Pathobiology, Univ Pennsylvania Sch Vet Med, Philadelphia, PA; 2) Dept Clinical Sciences, Univ Pennsylvania Sch Vet Med, Philadelphia, PA.

Mucopolysaccharidosis type IIIB (MPS IIIB) is a severe and lethal lysosomal storage disorder caused by a deficiency in activity of the enzyme *N*-acetyl-a-D-glucosaminidase (NAGLU), and subsequent lysosomal accumulation of the substrate heparan sulfate. To better utilize the canine model of MPS IIIB, we have identified the disease-causing mutation in the canine NAGLU gene.

Using the normal sequence of the canine NAGLU cDNA, primers were designed to amplify the genomic sequence of the canine NAGLU gene from the 5 to 3 UTRs. The genomic sequence of an affected dog was amplified and sequenced revealing the mutation to be a poly(A) insertion (~45 adenines) in the sixth exon of the NAGLU gene. The poly(A) insert is flanked on the 3 border by a 13 bp repeat of the native NAGLU sequence immediately 5 to the poly(A) insert. The insert was found to be polymorphic in the affected individual, differing by the number of adenine residues present. The insert is predicted to lead to the insertion of ~15 lysine residues starting after amino acid 704 of the unmodified protein sequence. Such a large insertion would likely disrupt normal enzyme activity, regardless of whether the number of adenine residues present lead to an in-frame insertion or not.

The presence of a poly(A) sequence and the flanking of the insert by a repeat of 5 native sequence at the 3 end of the insert, lead us to conclude that the insertion mutation was mediated by a LINE element. This would be the first recognition of an inherited disease causing mutation of this type in the dog, and the first in a species other than humans or mice. Supported by NIH grants RR002512 and RR007063, and by a grant from the National MPS Society, Inc.

**Familial mitochondrial myopathy: New insights into the T14709C mitochondrial tRNA mutation.** *A.M. Schaefer<sup>1</sup>, R. McFarland<sup>1</sup>, J.L. Gardner<sup>1</sup>, S. Lynn<sup>2</sup>, C.M. Hayes<sup>1</sup>, M.J. Barron<sup>1</sup>, M. Walker<sup>3</sup>, P.F. Chinnery<sup>1</sup>, R.W. Taylor<sup>1</sup>, D.M. Turnbull<sup>1</sup>.* 1) School of Neurology, Neurobiology and Psychiatry, The Medical School, Framlington Place, University of Newcastle upon Tyne, NE2 4HH, UK; 2) Dept. of Neurology and Neurological Sciences, Stanford University, Stanford, CA 94305; 3) School of Clinical Medical Sciences, The Medical School, Framlington Place, University of Newcastle upon Tyne, NE2 4HH, UK.

We have defined the genetic defect in a large family first described in one of the earliest reports of suspected mitochondrial myopathy, as the mutation T14709C in the mitochondrial tRNA<sup>Glu</sup> (mt-tRNA<sup>Glu</sup>) gene. Extraordinarily, this mutation has attained homoplasmy (100% mutated) on at least three independent occasions in this family and has done so in one individual who as yet remains asymptomatic and has no clinical evidence of disease. In all previous descriptions of the T14709C mutation, it has always been documented as heteroplasmic (co-existing wild type and mutated mitochondrial DNA [mtDNA]). Heteroplasmy is usually regarded as one of the primary diagnostic criteria for pathogenic mtDNA mutations but often does not provide an adequate explanation for phenotypic variation within families. Although a few homoplasmic mt-tRNA mutations have now been described, they have generally been regarded as rare and of low pathogenicity, resulting in organ-specific phenotypes such as deafness. Discovering that T14709C, a common and severe mt-tRNA mutation, can attain homoplasmy without symptoms or clinical signs of disease has profound implications for the identification and prevalence of other pathogenic mt-tRNA mutations. Furthermore, the observed variation in phenotype between individuals harbouring this homoplasmic mutation implies a crucial contribution from the nuclear genetic environment in determining the clinical outcome of mt-tRNA mutations.

**Amish microcephaly due to mitochondrial deoxynucleotide carrier deficiency is associated with lactic acidosis and agenesis of the corpus callosum.** *V.M. Siu, C.A. Rupa*. Depts of Pediatrics, Biochemistry and Pathology, University of Western Ontario, and CPRI, London, ON, Canada.

Amish microcephaly is an autosomal recessive mitochondrial disorder recently described in the Old Order Amish of Lancaster County. Reported features include severe congenital microcephaly with lissencephaly, hypoplastic pons and cerebellar vermis, death within the first year of life, and elevated urinary excretion of 2-ketoglutaric acid. Rosenberg (2002) found a homozygous mutation within the mitochondrial deoxynucleotide carrier (DNC) in affected infants. We report a male infant with microcephaly first noted at 20 weeks gestation. He was born at term weighing 3.3 kg with head circumference of 29 cm to consanguineous parents of Mennonite background. Amish microcephaly was suspected, based on MRI scan showing features described above. Agenesis of the corpus callosum was also present. However, urine organic acid analysis revealed no elevation of 2-ketoglutaric acid. At 5 months, he was admitted to hospital with failure-to-thrive and vomiting. Initial plasma lactate level was 6.7 mmol/L. While in hospital, he had 2 episodes of tachypnea and lethargy due to metabolic acidosis with plasma lactate rising to 16 mmol/L. Treatment with a mitochondrial metabolic cocktail and sodium bicarbonate resulted in lactate levels ranging between 8 to 10 mmol/L. Initiation of a modified ketogenic diet resulted in a dramatic decrease of plasma lactates to normal levels, accompanied by improved oral feeding, increased alertness, decreased hypertonicity and significant weight gain. Repeat urine organic acid analysis during metabolic acidosis failed to demonstrate elevated levels of 2-ketoglutarate, although urine lactate level was elevated. Mutation analysis of the DNC gene confirmed the same homozygous mutation as previously described in the Amish. We conclude that 2-ketoglutaricaciduria is not an obligatory finding and that lactic acidosis may be present in Amish microcephaly. We confirm the CNS malformations and add the feature of agenesis of the corpus callosum. Treatment with a ketogenic diet may help to correct lactic acidosis and potentially avert episodes of metabolic decompensation.

**Cerebral involvement in Mucopolysaccharidosis II: correlation between brain magnetic resonance findings and clinical manifestations.** *I.V.D. Schwartz<sup>1</sup>, L. Vedolin<sup>2</sup>, A.C. Puga<sup>1</sup>, L. Pinto<sup>1</sup>, F. Goncalves<sup>1</sup>, A. Pires<sup>1</sup>, R. Boy<sup>3</sup>, E.S. Santos<sup>3</sup>, I. Monllo<sup>3</sup>, M. Fontes<sup>3</sup>, E. Ribeiro<sup>3</sup>, C.A. Kim<sup>3</sup>, E. Valadares<sup>3</sup>, M. Damaso<sup>3</sup>, G. Carakushansky<sup>3</sup>, E. Kahn<sup>3</sup>, D. Norato<sup>3</sup>, R.O. Sobrinho<sup>3</sup>, R. Giugliani<sup>1</sup>.* 1) Medical Genetics Service, HCPA, Porto Alegre, Brazil; 2) Diagnostic Medicine, MDC, Porto Alegre, Brazil; 3) Brazilian MPS II Study Group.

Mucopolysaccharidosis type II (MPS II) is a rare lysosomal disorder caused by the deficiency of the enzyme iduronate L-sulfatase (IDS). To evaluate the effects of the IDS deficiency on the CNS, we studied 19 Brazilian MPS II male patients. These patients underwent brain MRI, as well as ophthalmologic, neurological and psychological evaluation, the latter including an IQ test. Mean age at evaluation was 9.3 ys (3-26 ys). 14/19 (74%) were classified as having the neurological form (N) and 5/19 (26%) the non-neurological form (NN). Isointense and hyperintense lesions were the most frequent findings in MRI, and were found in 16 patients (12/14 N, 4/5 NN); prominent cortical sulci were found in 12 (10/14 N, 2/5 NN), megacisterna magna was found in 7 (6/14 N, 1/5 NN), bilateral enlargement of the optic nerve sheath was found in 4 (3/14 N, 1/5 N), and hydrocephalus was found in 8 patients (8/14 N). Among patients with hydrocephalus, the most frequent clinical manifestation found was cognitive deficit (100%), followed by hyperreflexia (25%); papilledema and convergent strabismus were found in 1/8 patients. Alterations in spectroscopy were found in all patients, and are suggestive of axonal/white matter lesions. Our results suggest that brain isointense and hyperintense lesions, prominent cortical sulci, hydrocephalus, and megacisterna magna are frequent findings in MPS II. Among these findings, only hydrocephalus and megacisterna magna seem to be associated to the N form. Patients presented with hydrocephalus do not usually present signs/symptoms of acute intracranial hypertension (ICH), but they do present signs/symptoms of chronic ICH. Therefore, it is our understanding that the early diagnosis and treatment of ICH are essential for the preservation of the cognitive function of these patients (NORD/CAPES/TKT).

**Genetic variation of methylen tetrahydrofolate reductase gene in Iranian patients with coronary artery disease.**

*M. Ghaedi*<sup>1</sup>, *A. aleyassin*<sup>1</sup>, *S. Davoodi*<sup>2</sup>, *H. Abbasi*<sup>2</sup>. 1) National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran; 2) Tehran haert center, Shariati Hospital Tehran medical University, Tehran, Iran.

Several studies showed that elevated plasma homocysteine is a risk factor for coronary artery disease. common mutation C677T , A1298C of methylenetetrahydrofolate reductase (MTHFR) and A66G of methionine synthase reductase(MTRR) genes is reported to be associated with decreased enzyme activities and significant increased level of blood homocysteine. This study was to analyze the frequency of this mutation in 100 patients with CAD compared to the 100 normal control. It shows the higher prevalence of the C677T mutation in CAD patients compared to the normal control in Iranian tested patients. The C677T MTHFR was significantly linked to the CAD, supported by a P value <0.001 and Chi-square 51.82. Our observation showed the prevalence and significance of this mutation in studied cases compared to the normal control in Iranian cases.but the prevalence of the A1298C and A66G in CAD patients and normal control were equal. The A1298C MTHFR and A66G MTRR weren,t significantly linked to the CAD, supported by a P value 0.706 and chi-square 0.697 for A1298C and a Pvalue 0.052 and chi-square 5.923 for A66G mutations.However no significant corralation were obtained for A1298C and A66G MTRR mutation.

**MURR1: a new copper transport protein?** *L. Klomp<sup>1</sup>, P. de Bie<sup>2</sup>, A. Klomp<sup>1</sup>, B. van Oost<sup>3</sup>, C. Wijmenga<sup>2</sup>*. 1) Dept Pediatric Gastroenterolog, Wilhelmina Children's Hosp, Utrecht, Netherlands; 2) Dept Biomedical Genetics, University Medical Center Utrecht, the Netherlands; 3) Department of Veterinary Sciences, Utrecht University, the Netherlands.

Copper is an essential trace element for the survival of all organisms, although it is highly toxic above a certain threshold. To ensure proper copper homeostasis, copper import, distribution and export are well-regulated processes. Two homologous copper transport proteins have been identified which, when dysfunctional, cause either copper deficiency (Menkes disease) or copper accumulation (Wilson disease) in man. Non-Wilsonian hepatic copper toxicosis in man has been described to be phenotypically very similar to copper toxicosis (CT) in Bedlington terriers, which is a frequent genetic disease unique to this breed. We recently established that canine CT is due to a deletion encompassing exon 2 of MURR1. We also performed MURR1 mutation analysis in 23 patients with non-Wilsonian hepatic copper toxicosis. Although no mutation was found the MURR1 gene remains to be a candidate gene for other non-Wilsonian copper toxicosis patients. The function of the MURR1 gene is still unknown but we hypothesize that this gene is essential for biliary copper excretion downstream of ATP7B. Orthologs of MURR1 have been identified in a number of mammalian organisms, but could not be detected in lower eukaryotes and prokaryotes. MURR1 is most abundantly expressed in liver. Bedlington terriers with copper toxicosis accumulate copper in their lysosomes, suggesting that MURR1 is essential for excretion of copper into the bile. A polyclonal antiserum against MURR1 was successfully developed which showed that MURR1 is undetectable in the livers of affected Bedlington terriers. A yeast-two hybrid screen led to the identification of a yet unknown protein, which interacts with MURR1. A mouse knockout with a mutation comparable to the terriers is underway. Further characterization of MURR1, and its pathway, may lead to disentangling the complexities of copper metabolism in mammals and to the identification of new genes involved in copper homeostasis, which will be candidates for non-Wilsonian hepatic copper toxicosis.

**Human Acid Ceramidase: Catalytic Reactions and Interactions With Acid Sphingomyelinase.** *X. He, N. Okino, E. Schuchman.* Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY.

Human acid ceramidase (AC) is the enzyme deficient in the lysosomal storage disorder, Farber disease. AC was overexpressed in Chinese hamster ovary cells by amplification of the transfected, full-length cDNA. The majority of the overexpressed, human enzyme was secreted into the culture media and purified to homogeneity. The purified protein contained the same alpha and beta subunits as AC from natural sources, had an acidic pH optimum (4.5), and followed normal Michaelis-Menten kinetics. The purified enzyme also catalyzed ceramide synthesis using C12 fatty acid and sphingosine as substrates. In contrast to the degradative reaction, this reverse reaction occurred at an optimal pH of ~6.0. Cell extracts from patients with Farber disease had reduced reverse AC activity, confirming that the AC polypeptide was responsible for both reactions. Surprisingly, we also found that media from the overexpressing hamster cells had increased acid sphingomyelinase (ASM) activity. RNA studies showed that this increased activity was not due to overexpression of the endogenous ASM gene. Overexpression of AC in normal skin fibroblasts also led to enhanced ASM secretion, but this was not observed in Niemann-Pick disease cells. These studies provide new insights into AC and ASM, and suggest that these enzymes may exist within a multienzyme complex carrying out sphingolipid catabolism. These studies also reveal that patients with Farber disease have a defect in ceramide synthesis, as well as degradation.

**Lysosomal, but not Cytosolic Disulfide, Induces Apoptosis in Human Renal Proximal Tubule Cells.** *A.L. Jones, M.A. Park, J.G. Thoene.* Human Genetics, Tulane University, New Orleans, LA.

We have previously shown that renal proximal tubule epithelial (RPTE) cells show a ten-fold increase in apoptotic rate when exposed to cystine dimethylester (CDME), a compound that causes accumulation of cystine within lysosomes (Park et al, 2003). It is not yet known whether lysosomal localization of the disulfide is required for enhanced apoptosis to occur. We here report that RPTE cells do not show increased apoptosis when treated with equimolar amounts of compounds that do not result in lysosomal disulfide localization. The basal apoptosis rate in these cells is 1.8% (CaspACE and FACS). CDME exposure results in an increased apoptosis rate to 37.0%. The two non-esterified disulfides, cystamine and penicillamine disulfide resulted in 2.5% and 2.4% apoptosis respectively after the cells were exposed to 0.5mM compound for 6 h. The non-disulfide congener of cystine, djenkolic acid caused only 1.9% apoptosis when added to the RPTE cells for the same period at the same concentration. The fluorescent dye, JC-1 is known to reflect the redox state of mitochondria. Exposure of cultured fibroblast cells to CDME for 12 hr at a concentration of 0.5 mM, produced an increase in the proportion of positive cells from 11.7% to 36.6%. We conclude that lysosomal cystine increases apoptosis via a perturbation in the redox state of the cell, which is mediated by lysosomal release of disulfides.

**Mouse Skin Fibroblasts and Renal Proximal Tubule Cells Do Not Display Lysosomal Cystine Enhanced Apoptosis.** *M. Park, A. Jones, J. Thoene.* Dept Human Genetics, Tulane Univ, New Orleans, LA.

Cystinosis is a disease of lysosomal cystine storage characterized by short stature, corneal clouding, renal Fanconi syndrome, and end-stage renal failure by age ten years if untreated. It results from a mutation in the gene CTNS, which codes for the lysosomal cystine transporter. We have recently shown that lysosomal cystine storage causes human fibroblasts and renal proximal tubule cells to display 2-4 fold increased apoptosis (Park et al, J Am Soc Nephrol. 2002; 13, 2878-2887). Cherqui et al (Mol Cell Biol. 2002; 22, 7622-7632), described the first cystinosis knock out mouse. Increased cystine was detected in all tissues observed, however, the mice do not express the renal phenotype seen in man. Human renal proximal tubule epithelial (RPTE) cells treated with cystine dimethylester (CDME, a compound that causes lysosomal cystine storage) yield an apoptosis rate of 88.0% (baseline 2.5%), while primary mouse RPTE cells so treated display an apoptotic rate of only 22.8% (baseline 10.1%). Similar results were obtained with human and mouse skin fibroblasts: Human transformed fibroblast cell line GM00637 displayed an apoptotic rate of 49.9% 9.7 after CDME treatment (baseline 4.9% 2.08), contrasted with mouse L929 fibroblasts, that had an apoptotic rate of 5.8% 0.60 after CDME (baseline 6.3% 2.5) ( $p=0.01$ ). A generalized hypocellularity of critical organs and tissues due to enhanced apoptosis mediated by lysosomal cystine storage can explain the cystinotic phenotype. Failure to display this lysosomal cystine-enhanced apoptosis may account for the failure of the CTNS knockout mouse to faithfully model the human disease cystinosis.

**A new mutation in the EIFAK3 gene, encoding the transmembrane endoplasmic reticulum (ER) kinase PERK, in a patient with Wolcott-Rallison syndrome (WRS).** *F. Durocher<sup>1</sup>, Y. Labrie<sup>1</sup>, L. Pelletier<sup>2</sup>, M.B. Plourde<sup>1</sup>, R. Laframboise<sup>2,3</sup>, R. Faure<sup>2</sup>.* 1) Cancer Genomics Laboratory,; 2) Pediatrics Research Unit; 3) Medical Genetics, CHUL Research Centre, CHUQ, Laval University, Quebec, Canada,;

Wolcott-Rallison syndrome (WRS) is a rare autosomal recessive disorder characterized by permanent neonatal insulin-dependent diabetes, severe epiphyseal dysplasia, osteoporosis and growth retardation. Clues to the regulation of protein synthesis in secretory cells have recently come from the investigation of the unfolded protein response (UPR), an adaptive mechanism which controls the deleterious effects of accumulating unfolded proteins in the ER. The transmembrane kinase EIF2AK3/PERK functions by inhibiting protein synthesis following the dissociation of chaperones associated from its intraluminal domain. In two previous studies, mutations in the EIF2AK3/PERK gene, which encodes a protein of 1115 amino acids, have been found in WRS patients. We report here the finding of a novel nonsense mutation in a French Canadian family. Sequencing analysis of the proband revealed the presence of a homozygous G to T change in exon 5 leading to the nonsense E331X mutation (GAG/TAG). The mutation therefore produces a largely truncated protein of 330 amino acids in which the cytosol-oriented domain of PERK, containing the kinase domain, located from amino acid 577 to 1115 in the wild-type protein, is missing. Both parents were found heterozygous for the same mutation. No other case was present in the family. These results confirm the role of EIF2AK3/PERK in WRS and its utility for diagnoses. The results also further point out the role of intracellular organelles in the control of signaling pathways and physiological glucose homeostasis.

**Clinical and molecular findings in a patient with Griscelli syndrome.** *M. Gunay-Aygun<sup>1</sup>, A. Dobbie<sup>2</sup>, R. Kleta<sup>1</sup>, H. Dorward<sup>1</sup>, Y. Crow<sup>2</sup>, W.A. Gahl<sup>1</sup>, P. McClean<sup>2</sup>, M. Huizing<sup>1</sup>.* 1) Section on Human Biochemical Genetics, MGB, NHGRI, NIH, Bethesda, MD; 2) St James's Hospital, Leeds, United Kingdom.

Griscelli syndrome (GS) is an autosomal recessive disorder characterized by partial albinism (hypopigmented skin and silvery hair) with immunodeficiency and onset of neurological impairment secondary to hemophagocytosis involving the central nervous system. Mutations in *Rab27A*, which codes for a small GTPase involved in vesicle trafficking, lead to GS, while mutations in *Myo5A*, which codes for a myosin interacting with both intracellular vesicles and actin filaments, result in Elejalde syndrome (ES). Both disorders share clinical signs and symptoms, but in ES the neurological involvement is primary and no immune defects occur. Initially it was thought that both these closely localized genes on 15q21 caused GS. Here we present the clinical course of an offspring of first cousins affected by GS who died at the age of 4 years from infection-associated complications. The patient presented at the age of 3 years with recurrent, 2-3 week long episodes of fever associated with splenomegaly, pancytopenia, episcleritis, and a skin rash. The patient had silver grey hair and was small for age. A liver biopsy showed features of advanced chronic hepatitis, and serum titers indicated hepatitis B. IgG levels were persistently elevated. A skin biopsy showed chronic inflammatory cells within the dermis. At age 3 years, the patient developed left hemiparesis during an episode of fever. Brain imaging showed a large porencephalic cyst in the right frontal region with some white matter edema. No vascular malformations could be documented. Bone marrow aspirate, obtained during a healthy period, showed no signs of hemophagocytosis. Sequencing of all coding exons of *Rab27A* verified the clinical suspicion of GS by demonstrating a new homozygote splice site mutation in intron 3, i.e., IVS3-2 A>G. In summary, this patient exhibited clinical signs and symptoms of GS with secondary neurological abnormalities. Differentiating between *Rab27A* and *Myo5A* disorders is important for the clinical management and care of affected patients.

**Fabry disease: Recognition and characterization of the renal variant.** *C. Carter, J. Wiszniewska, B. Roa, C.M. Eng.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Fabry disease, an X-linked deficiency of the lysosomal hydrolase, alpha-galactosidase A, results in the accumulation of glycosphingolipids, primarily GL-3, in the vascular endothelium and other cell types in the body. Classically affected males manifest the typical signs and symptoms of the disorder, i.e., angiokeratoma, acroparesthesias, corneal dystrophy, progressive involvement of the kidneys, heart, and cerebrovascular systems, and follow a predictable disease course. In contrast, several variant presentations of Fabry disease have been recognized that are characterized by milder, sometimes organ specific dysfunction. The previously described cardiac variant consists of individuals who lack the classic signs and symptoms of the disease and have disease manifestations mostly limited to the heart. Recently, several individuals have been identified who lack the classic signs and symptoms, but have renal manifestations that parallel those of classically affected males. A 42 year old male presented with proteinuria (796 mg/24 hr) and renal insufficiency (serum creatinine 1.4 mg/dl). A renal biopsy showed ultrastructural features consistent with Fabry disease and leukocyte alpha-galactosidase A activity was deficient (4 nmoles/hr/mg; nl range 55-126). The patient denied a history of acroparesthesias or hypohidrosis. Exercise tolerance was good. Physical exam revealed no angiokeratoma or corneal dystrophy and cardiac examination was normal. DNA sequencing of the entire alpha-galactosidase A coding region and flanking intronic sequences demonstrated a single nucleotide substitution resulting in the N215S genotype. This genotype was previously identified in several unrelated cardiac variant patients as well as classically affected individuals. Recognition of the variant presentations of Fabry disease adds to the spectrum of disease manifestations that can present in this disorder. The phenotypic spectrum associated with the N215S genotype continues to broaden. The cardiac and renal variants, as well as symptomatic heterozygotes present a diagnostic challenge, however prompt recognition and consideration of treatment may improve the long term outcome.

**Genotype and phenotype analysis in Wilson disease.** *D.W. Cox*<sup>1</sup>, *L.M. Prat*<sup>1</sup>, *J.M. Walshe*<sup>2</sup>, *E.A. Roberts*<sup>3</sup>, *L.M. Cullen*<sup>1</sup>. 1) Medical Genetics, Univ Alberta, Edmonton, AB, Canada; 2) Hemingford Grey, UK; 3) Hospital for Sick Children, Toronto, ON.

Wilson disease (WND), a disorder of copper transport, is extremely variable in type of manifestation (hepatic and/or neurologic) and age of onset (3-50 or more yrs). We carried out mutation analysis on 173 patients diagnosed with WND by clinical, biochemical and molecular analysis: 146 of various European origins (91 from Canada, 55 from UK), 10 Canadians of Chinese origin, and 17 Indian or Pakistan origin from Canada and the UK. Mutation analysis was carried out by assay of the common His1069Gln mutation and sequencing of 10 or more exons of the 21 exons of *ATP7B*. In the European origin group, at least one mutation was identified in 91% of patients: of these mutations, 36% were H1069Q, 17% in exon 8, 8% in each of exons 13 and 18, and less than 5% in other exons. In the total group, 79% were missense, 16% small deletions or insertions, 2% nonsense, and 3% splice site mutations. In comparison, distribution of 262 mutations listed in the WND Mutation Database ([www.uofa-medical-genetics.org](http://www.uofa-medical-genetics.org)) in these classes is 58, 27, 7 and 8%; respectively. A promoter mutation was found only in patients of Sardinian origin. The exon distribution of mutations was similar for neurological and hepatic onset. Type of mutation influences age and type of onset of the disease. Among 60 homozygotes for the same mutation from all ethnic groups, the most informative for phenotype/genotype correlation, age of onset was 11.4 yrs. for those with severe mutations (deletions, insertions, nonsense), with 11 hepatic and 4 neurologic onset. Early onset was usually with hepatic onset. For 16 with various missense mutations, age of onset was 17.8 yrs. The common His1069Gln mutation had an age of onset of 20.3 yrs. In the European group, age of onset was influenced by the class of the mutation: 11.8 yrs for severe homozygous mutations, 18.7 for various combinations of missense homozygotes. Missense mutations need study by functional assays to ensure they are not normal rare variants. Age of onset is due in part to the severity of the mutation present, but other modifying factors appear to be important.

**Role of genetic modifiers in determining the phenotype in Gaucher disease: A closer look at the genotype**

**L444P/L444P.** *E. Sidransky*<sup>1, 2</sup>, *E. Orvisky*<sup>1, 2</sup>, *A. Lwin*<sup>1, 2</sup>, *B. Stubblefield*<sup>1, 2</sup>, *R. Schiffmann*<sup>3</sup>, *O. Goker-Alpan*<sup>1, 2</sup>. 1) NSB/NIMH/NIH, Bethesda, MD; 2) MGB/NHGRI, NIH, Bethesda, MD; 3) DMNB/NINDS, NIH, Bethesda, MD.

Gaucher disease (GD), the inherited deficiency of lysosomal glucocerebrosidase, is classified into three major clinical types depending upon the degree of nervous system involvement. Type 3 GD is a progressive neuronopathic form, and is often associated with severe systemic disease. Homozygosity for the common L444P allele usually correlates with neuronopathic GD. However, previous genotype/phenotype studies indicate that there can be vast phenotypic variation even among patients sharing the same genotype. To define the spectrum of clinical heterogeneity and elucidate the role of genotype and/or modifier genes in defining phenotype, twenty-four children (10M: 14F) with genotype L444P/L444P were identified. Genotyping was performed by both direct sequencing and Southern blots, confirming the absence of a recombinant allele. The residual glucocerebrosidase activity, measured in fibroblast cell lines, ranged from 1-20% and did not correlate with clinical presentation. The phenotypes encountered varied from death in early childhood to relatively asymptomatic college students. The age at diagnosis ranged from 2 months to 2 years, with an average of 15 months. Eighteen patients received enzyme replacement therapy (ERT) and six died by adolescence before ERT was available. The systemic involvement was most severe in the cohort who had undergone splenectomy without receiving ERT. Clinically, all appeared to have isolated supranuclear palsy. Two children had clinical seizures, while most had abnormal EEGs. Less than 20% of patients had developmental delay. Even though ERT significantly decreased the morbidity and mortality, the broad phenotypic variation among L444P homozygotes was still accounted for only in part by therapeutic intervention. These findings implicate the contribution of genetic modifiers in determining phenotype in GD.

**Pseudoxanthoma elasticum development of a mouse model by targeted ablation of ABCC6.** *J. Uitto, Y. Matsuzaki, J. Terlizzi, K. Li, D. Leperi, J. Klement, L. Pulkkinen.* Dermatology/Cutaneous Biol, Thomas Jefferson Univ, Philadelphia, PA.

Pseudoxanthoma elasticum (PXE), an autosomal recessive disorder, is characterized by late-onset calcification of pleiotropic elastic structures in the skin, eyes, and the cardiovascular system. The mutated gene was recently shown to be ABCC6 encoding MRP6, a transmembrane protein of unknown function, expressed primarily in the liver. We have embarked on development of a mouse model for PXE using targeted ablation of the ABCC6 gene. The targeting construct replaced 6.9 kb of genomic sequence resulting in elimination of exons 15-18 from the corresponding mRNA (545 bp), which is out-of-frame predicting a null allele. Southern analysis and genomic PCR showed the final products to be homozygous for the deletion in the ABCC6 gene. Northern analysis of polyA<sup>+</sup> RNA from the liver revealed that the mutant ABCC6 mRNA was not expressed, suggesting that it is unstable. Staining of liver from wild-type mice with antibody anti-MRP6 antibody revealed membrane-associated baso-lateral staining, while the staining was entirely negative in the ABCC6<sup>-/-</sup> mice. These findings confirm that the mice are truly null with regard to MRP6 expression. Examination of elastic structures by histochemical stain (Verhoeff-van Gieson) and by immunohistochemistry with anti-elastin antibody revealed that the elastic structures in the eye (Bruch's membrane), in the skin, and in the aorta were apparently normal in ABCC6<sup>-/-</sup> mice at eight weeks of age, and von Kossa staining for calcium was negative. These findings suggest that the development of the elastic fiber network in tissues affected in PXE is normal in these mice, at least during the early stages of development. Examination of skin at 32 weeks suggested that the elastic fibers were sparse and fragmented in ABCC<sup>-/-</sup> mice, but there was no evidence of aberrant calcification. Thus, the findings in the ABCC6 knock-out mice are consistent with late-onset of human PXE.

**Lysosomal Disease Clinical Research Center WORLD.** *E. Shapiro*<sup>1</sup>, *L. Elsas*<sup>2</sup>, *C. Eng*<sup>3</sup>, *R. Martin*<sup>4</sup>, *D. Marsden*<sup>5</sup>, *G. Pastores*<sup>6</sup>, *R. Steiner*<sup>7</sup>, *W. Wilcox*<sup>8</sup>, *S. Winter*<sup>9</sup>, *C. Peters*<sup>1</sup>, *J. Hegland*<sup>1</sup>, *D. Erickson*<sup>1</sup>, *L. Charnas*<sup>1</sup>, *C. Whitley*<sup>1</sup>. 1) Univ Minnesota, Minneapolis; 2) Univ Miami, Miami; 3) Baylor College of Medicine, TX; 4) Washington Univ, St. Louis, MO; 5) Harvard Univ, Boston, MA; 6) New York Univ, New York, NY; 7) Oregon Health Sciences Univ, Portland, OR; 8) Cedars-Sinai Med Center, Los Angeles, CA; 9) Children's Hosp Central California, Madera, CA.

The Lysosomal Diseases Clinical Research Center WORLD is a scalable multi-center consortium of geographically distributed expert medical centers, together planning and promoting clinical research on rare lysosomal diseases, i.e., mucopolysaccharidoses, glycosphingolipidoses, oligosaccharidoses and ceroid lipofuscinosis. The network is developing longitudinal studies to understand the natural history of health, neuropsychological, and quality-of-life outcomes, with and without treatment. The first longitudinal study focuses on the natural history of MPS I and treatment effects of hematopoietic cell transplantation (HCT) and enzyme replacement (ERT). The linked multi-center approach facilitates the development of common criteria, protocols, nomenclature and methods that will have broad applicability. On an annual basis, the WORLD Advisory Board supports 2 to 6 "pilot" or "demonstration" projects aimed at 1-year hypothesis-driven studies exploiting the combined resources of the center. Four studies for this year are: 1) Introduction of MPS Screening in a Multicultural Medical System, 2) ERT in Conjunction with HCT for MPS I, 3) Ventilatory and Cardiovascular Abnormalities at Rest and During Exercise in Fabry Disease, and 4) Adult/Late-onset Tay-Sachs Disease: A Model for a Rare Lysosomal Storage Disease and Inherited Motor Neuron Disease. The WORLD training program supports a broad group of activities including an annual scientific and educational meeting (May 13-16, 2004), site visits to each of the centers, and fellowships to enhance new clinician-investigators that are needed in the field. A website, [www.world.umn.edu](http://www.world.umn.edu), provides education and training resources for professionals, on-line data management, and information for the lay population.

**Nevo syndrome or kyphoscoliotic type of Ehlers-Danlos syndrome (EDS VIA)?** A. Randolph<sup>1</sup>, C. Giunta<sup>1</sup>, L.I. Gazali<sup>2</sup>, M.E. Kraenzlin<sup>3</sup>, B. Steinmann<sup>1</sup>. 1) Division of Metabolism & Molecular Pediatrics, University Children's Hospital, CH-8032 Zurich, Switzerland; 2) Department of Pediatrics, UAE University, Al Ain, United Arab Emirates; 3) Division of Endocrinology and Diabetology, University Hospital, CH-4031 Basel, Switzerland.

The Nevo syndrome (MIM 601451) is a rare autosomal recessive disorder characterized by overgrowth, muscle hypotonia, joint laxity and kyphoscoliosis. Since its first description in 1974 by Nevo *et al.* only a few cases have been reported. In our opinion some of these patients present clinical features of EDS VIA (MIM 225400), an inherited connective tissue disorder characterized by a deficiency of lysyl hydroxylase due to mutations in *PLOD1*. This enzyme deficiency results in underhydroxylation of lysyl residues and underglycosylation of hydroxylysine residues of collagens and, hence, to an abnormal pattern of pyridinoline crosslinks in the urine. We report on two patients published by Al-Gazali *et al.* (1997) and on an additional case also from an inbred family of United Arab Emirates. The ratio of total urinary lysyl pyridinoline to hydroxylysyl pyridinoline was elevated (3.5 and 8.2) compared to controls (0.20-0.10, range 0.10-0.38) and similar to those in EDS VIA (5.97-0.99, range 4.3-8.1, n=17). Genomic DNA was extracted from blood and the 19 exons of *PLOD1* were amplified by PCR followed by direct sequencing. In the three families, we found that both parents were heterozygous for a point mutation in exon 9 of the *PLOD1* gene, *i.e.* g.23939CGA>TGA (c.954CGA>TGA) which causes a R319X nonsense mutation. The affected offspring in each family was homozygous for the same mutation which has been shown to cause EDS VIA (Hyland *et al.*, Nat Genet, 1992). In addition, a new polymorphic variant (SNP) has been identified in intron 13 for all three families (g.30111G>A) as well as a new SNP in exon 3 for two of the three families (g.15210C>T). We have not yet established if one or both of these SNPs are associated with the mutation.

We conclude that at least some of the reported patients with Nevo syndrome have EDS VIA instead. It will be interesting to corroborate this finding in additional Nevo syndrome patients.

**An Atypical Case of Smith-Lemli-Opitz Syndrome, Both Clinically and Biochemically.** *D. Kostiner*<sup>1</sup>, *L. Merkens*<sup>2</sup>, *K. Crow*<sup>1</sup>, *R. Foxley*<sup>1</sup>, *W. Connor*<sup>4</sup>, *R. Steiner*<sup>2,3</sup>. 1) Genetics, Kaiser Permanente PC, Portland, OR; 2) Pediatrics, Oregon Health & Sci Univ; 3) Mole&Med Genetics, OHSU; 4) Medicine, OHSU.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder caused by mutations in the gene encoding the final enzyme in cholesterol synthesis, 7-dehydrocholesterol delta 7 reductase (*DHCR7*). High levels of plasma 7-dehydrocholesterol (7DHC) and 8-dehydrocholesterol (8DHC) are usually diagnostic for SLOS. We present a baby with unusual phenotypic features who was diagnosed with SLOS based on biochemical analysis and mutation analysis. The phenotype was more severe than predicted by the sterol levels and not very suggestive of SLOS. We suspect the baby has an atypical form of SLOS or a second syndrome that is unrelated. The baby was born at 30 weeks gestation to a 16-year-old G1P0-1 mother via c-section for preeclampsia. She was hospitalized 33 days for poor growth, then readmitted for failure to thrive, respiratory illness, and unexplained hyperkalemia. Exam revealed Ht & Wt <3rd%ile; HC <<3rd%ile; dysmorphic facies; high palate; long fingers with flared tips; 5th finger clinodactyly; short halluces; thin skin; prominent veins; and sparse hair. Workup showed normal results for karyotype (46,XX); cystic fibrosis mutations; biotinidase; brain CT/MRI; EEG; renal US; and hair analysis. SLOS was considered based on hyperkalemia, FTT, microcephaly, and short halluces. Plasma sterol concentrations (mg/dL) showed cholesterol of 92; 8DHC of 0.1 (nml 0); and 7DHC of 0.15 (nml 0-0.3). In our experience, the lowest plasma 7DHC range for mildly affected SLOS patients is 0.3-0.9. Mutation analysis showed 2 mutations in the *DHCR7* gene: 452GA; W151X, and an unpublished mutation 1381CT; R461C. Analysis of the baby's parents showed that the mutations were not on the same allele. We conclude: 1. Children with plasma 7DHC as low as 0.1 mg/dL could be affected with SLOS. Mutation analysis or *DHCR7* enzyme activity levels may be needed for definitive diagnosis in atypical cases. 2. Unusual phenotype (thin skin, prominent veins, sparse hair, short great toes) may be due to an SLOS variant related to the novel mutation, or perhaps to a second syndrome, such as an ectodermal dysplasia.

**CLINICAL STUDY OF 10 BRAZILIAN CASES OF MUCOPOLYSACCHARIDOSIS VI (MAROTEAUX-LAMY SYNDROME).** *C.A. Kim<sup>1</sup>, A.C. Paula<sup>1</sup>, D.R. Bertola<sup>1</sup>, L. Toma<sup>2</sup>, R. Giugliani<sup>3</sup>, M.B. Moreira<sup>1</sup>, V.H. Ferraro<sup>1</sup>, L.M.J. Albano<sup>1</sup>.* 1) Dept Pediatrics, Inst da Crianca, Sao Paulo, Brazil; 2) Disc Biologia Molecular, UNIFESP; 3) Genetica e Bioqumica, UFRGS.

Mucopolysaccharidosis type VI (MPS VI), also known as Maroteaux-Lamy syndrome, is an autosomal recessive lysosomal storage disorder caused by the deficiency of the lysosomal hydrolase, N-acetylgalactosamine-4-sulfatase, formerly known as arylsulfatase B, responsible for the degradation of dermatan sulfate. There are three recognized clinical entities: mild, moderate and severe. The evolution is progressive with multisystem involvement, specially skeletal, cardiac and pulmonary. We report on 10 Brazilian patients with MPS VI. The mean age at diagnosis was 10y 5mo (ranging from 2 years to 19 years) and their current age ranged from 7y 5mo to 19y 11mo. Parental consanguinity was observed in 2/8 families (25%). All patients presented coarse facies, short stature, hepatomegaly, joint stiffness, gibbous vertebrae/kyphosis and normal intelligence. Others findings: cardiac abnormalities (90%), hirsutism (80%), claw-hands (80%), corneal opacity (70%), macrocephaly (60%), motor delay (20%) and speech delay (10%). Evolution: respiratory infections (50%), sleep apnoea (30%), reversible cardiac arrest (30%), surgery (30%), visual deficit (20%), hydrocephaly (10%) and insuccess to oral intubation (10%). Three patients died of heart failure and respiratory distress due to airway obstruction (major survival 21y10mo). The progressive course of the disease and the possibility of enzyme replacement therapy, make an early diagnosis imperative for a better management of these patients with MPS VI.

**Normative data in adult female patients with Fabry disease.** *R. Schiffmann, S. Gupta.* Disorders & Stroke, DMNB/Natl Inst Neurological, Bethesda, MD.

Fabry disease (alpha-galactosidase A deficiency) is an X-linked disorder leading to accumulation of glycosphingolipids, predominantly globotriaosylceramide (GL-3) in cellular lysosomes. Females may be affected due to random inactivation of one X-chromosome. This 7-day prospective study was undertaken to define normative data in female Fabry patients. Fifty-seven female patients were confirmed by genotyping to have Fabry disease. Mean (SD) age was 43 (12) years. The mean age at onset of symptoms was 21 years (n=38). Mean plasma GAL activity was 3.2 nmol/hour/mL (ranged from 1.5-15.9; normal 2.4-23.1 nmol/hour/mL), and mean leukocyte GAL activity was 28.2 nmol/hour/mg (ranged from 4.0-76.7; normal 46-126.4 nmol/hour/mg). Notable specific Fabry-associated medical history included acroparesthesia (74%), cornea abnormalities (67%), angiokeratomas (63%), and pain (58%). Most patients (88%) felt their pain interfered with their daily activities, and 29 (51%) patients took rescue pain medication. Mean urinary GL-3 (331.8 nmole CTH/g creatinine/24 hours) was elevated compared with normal female patients (5.61.0 nmole CTH/g creatinine/24 hours, n=3). Mean and median plasma GL-3 (6.4 and 6.3 g/mL, range 2.1-10.6 g/mL) were normal. Serum creatinine was normal (mean 0.8 mg/dL) for most patients. Estimated glomerular filtration rate (MDRD study equation) was low ( $<90$  mL/min/1.73m<sup>2</sup>) in 24 (42%) patients. Left ventricular hypertrophy and conduction abnormalities were observed in 18% and 26% of patients, respectively, on EKG. Mitral and tricuspid valve regurgitation were the most common echocardiographic abnormalities (each in 75% of patients). Four patients (7%) had a cerebral infarction, and 10 (18%) patients had an abnormal MR angiogram. Minimal accumulations of GL-3 were found in capillary endothelial skin cells. A gender difference in plasma GL-3 levels is evident, and female Fabry patients do not have low plasma and leukocyte GAL levels as observed in males. Genotyping should be the gold standard for diagnosis of Fabry disease among females. This study confirms the very significant, yet heterogeneous, burden of disease in female Fabry heterozygotes.

**Urine glycosaminoglycan measured with the MPStest is a convenient means of monitoring response to treatment, and may be an indicator of clinical phenotype.** *G.S. Sahi, C.M. Erickson, D.C.C. Erickson, N.Q. Lam, S. Lo, C.B. Whitley.* Institute of Human Genetics, University of Minnesota, Minneapolis, MN.

Urine specimens are easily obtained and transported for determination of glycosaminoglycan (GAG) using the *MPStest*. To explore the potential use of this test in the diagnosis and treatment of mucopolysaccharidosis (MPS) diseases, this test was applied in several clinical situations. Specimens demonstrated stability for more than 10 years when stored under ambient conditions without special processing. In patients undergoing a variety of treatments, decreasing GAG/creatinine paralleled other markers of response to therapy, generally corresponding to early indicators of clinical outcome. For example, urine GAG/creatinine decreased in patients demonstrating successful donor engraftment after bone marrow transplantation. In a case of experimental lymphocyte gene therapy for Hunter syndrome, there was no change in GAG/creatinine, a result corresponding to the lack of other clinical indicators of efficacy or surrogate markers. Patients undergoing enzyme replacement therapy have been studied by other, more laborious methods (requiring collection of a 24-hour urine specimen); results from the *MPStest* methodology are being accumulated. Among a limited number of patients with MPS disease, there appears to be a correspondence of urine GAG/creatinine to "severity" of the disease, thus suggesting that urine GAG/creatinine might be useful as a prospective indicator of clinical severity, a postulate under further investigation.

Program Nr: 1656 from 2003 ASHG Annual Meeting

**The MPS I Registry.** *J.T.R. Clarke<sup>1</sup>, G.M. Pastores<sup>2</sup>, D. Viskochil<sup>3</sup>, for the International MPS I Registry Board of Advisors.* 1) Hospital for Sick Children, Toronto, Canada; 2) NYU, NY, NY; 3) U of Utah, Salt Lake City, Utah.

**Overview:** Systematically collected, longitudinal data on large numbers of patients with MPS I, a rare, autosomal recessive mucopolysaccharide storage disorder, are necessary for the rigorous long-term evaluation of any specific treatment for the disease. An international MPS I Registry has been established to provide the mechanism for the collection, storage, and dissemination of this information. **Objectives/Methods:** The objective of the Registry is to systematically enroll patients with the disease and collect clinical information on the natural history, as well as to track the long-term outcomes of treatment with Aldurazyme (laronidase). Over the past 18 months, an International Board of Advisors, working in collaboration with the sponsors, have been developing comprehensive Case Report Forms (CRFs) to support the collection of relevant data on the natural history of MPS I, including clinical events, course of treatment, and related outcomes. **Results:** Drafts of the CRFs for use by participating physicians were extensively reviewed. Annual reports from this Registry will be submitted to the regulatory agency, in part to fulfill post-approval commitments, as well as providing a database for additional sub-studies on specific aspects of MPS I. The infrastructure for implementation is provided by BioMarin/Genzyme LLC. The Registry is modelled after a similar database, developed over 10 years ago by the International Collaborative Gaucher Group (ICGG), for monitoring patients with Gaucher disease. Using well-validated measures of disease pattern and severity, information will be collected on physical and functional well-being, as well as the performance of activities of daily living, to evaluate benefits derived from various therapeutic interventions, including enzyme replacement therapy. **Conclusion:** As uniform data are successfully collected on large numbers of patients with MPS I, the characterization of disease course should enable identification of early predictors and determinants of morbidity and mortality. Close monitoring and the data collected will provide the basis of the assessment of the impact of therapy. (Sponsored by BioMarin/Genzyme LLC).

**Fabry disease: Delineating the natural history of the disorder through the Fabry Registry.** *C.M. Eng<sup>1, 2</sup>, M. Banikazemi<sup>2</sup>, J. Barranger<sup>2</sup>, J. Charrow<sup>2</sup>, L. Clark<sup>2</sup>, D. Bushinsky<sup>2</sup>, R. Hopkin<sup>2</sup>, G. Pastores<sup>2</sup>, C.R. Scott<sup>2</sup>, K. Sims<sup>2</sup>, W. Wilcox<sup>2</sup>.* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) North American Board of Medical Advisors, Fabry Registry.

Fabry disease, an X-linked deficiency of alpha-galactosidase A, results in the accumulation of glycosphingolipids, primarily globotriaosylceramide (GL-3) in the lysosomes of the vascular endothelium as well as other cell types and tissues throughout the body. In general, affected males demonstrate the classical signs and symptoms of the disease, including progressive end organ involvement of the renal, cardiovascular, and central nervous systems. Female heterozygotes have a variable clinical course with disease manifestations ranging from minimal to the full spectrum of clinical findings seen in males. As is typical with orphan disorders, a full understanding of the clinical course and rate of disease progression has been limited by the lack of prospectively obtained data on a significantly large population of affected individuals. To address these issues, a web-based, centralized registry and database was developed ([www.fabryregistry.com](http://www.fabryregistry.com)). In the early stages of this effort, 178 patients (65% male and 35% female) have consented to join the study and their data underwent preliminary analysis. Ninety percent of participants were over the age of 18 years with the median age being 43. The median age at diagnosis was 29.5 and the median age of onset of disease symptoms was 9 years (n=116). At the time of diagnosis, symptoms in the following organ systems were the most commonly cited as the presenting symptom: neurological (pain) (48%), skin (angiokeratoma) (29%), gastrointestinal (17%), and renal (16%). In determining the order of onset of end organ clinical events, a trend toward renal events preceding cerebrovascular and cardiac events was observed. The analysis of clinical information from a large patient population will greatly enhance our understanding of the natural history of Fabry disease in both males and females as well as facilitate the development of monitoring protocols to assess the effectiveness of therapeutic interventions, particularly, enzyme replacement therapy.

**Positive European experience with agalsidase alfa in Fabry disease: update from FOS - the Fabry Outcome Survey.** A. Mehta<sup>1</sup>, M. Beck<sup>2</sup>, F. Dehout<sup>3</sup>, A. Garcia de Lorenzo<sup>4</sup>, G. Hauge<sup>2</sup>, P. Jaeger<sup>5</sup>, C. Kampmann<sup>2</sup>, A. Linhart<sup>6</sup>, R. Ricci<sup>7</sup>, G. Sunder-Plassman<sup>8</sup>, U. Widmer<sup>9</sup>, on behalf of the FOS investigators. 1) Dept of Haematology, Royal Free Hosp, London, UK; 2) Dept of Pediatrics, Univ of Mainz, Germany; 3) Dept of Nephrology, CHU de Charleroi, Belgium; 4) Formación Médica Continuada Hospital Univ, Madrid, Spain; 5) Dept of Nephrology, Hôpital Pasteur, Nice, France; 6) 2nd Dept of Internal Medicine, Charles Univ, Prague, Czech Republic; 7) Institute of Clinical Pediatrics, UCSC Rome, Italy; 8) Division of Nephrology and Dialysis, Univ of Vienna, Austria; 9) Dept of Medicine, Univ of Zurich, Switzerland.

Fabry disease is a rare X-linked lysosomal storage disorder caused by  $\alpha$ -galactosidase A deficiency. This results in progressive lysosomal storage of globotriaosylceramide (Gb3) in various cells throughout the body. Both men and women are affected, with symptoms developing in childhood and premature death occurring usually from renal failure, cardiac disease or cerebrovascular complications. Enzyme replacement therapy (ERT) has now been available in Europe for 2 years. FOS is the largest database for patients with Fabry disease, and contains data on some 400 patients treated with, or candidates for treatment with, agalsidase alfa (Replagal; TKT-5S, Danderyd, Sweden). Of the 65% of patients given agalsidase alfa in FOS, 70% have now been treated for 1 year. Significant beneficial effects of agalsidase alfa treatment have been reported in FOS. Pain, one of the major and early clinical manifestations of the disease, is reduced, as assessed by the Brief Pain Inventory (p0.001), and overall quality of life improved (p0.05). In addition, cardiac function, measured by the QRS duration and Sokolov-Lyon product, is improved (p0.05), consistent with reductions in left ventricular mass. Furthermore, agalsidase alfa can stabilize renal function and even reverse the decline in renal function in patients with a GFR of 60-89 ml/min (p=0.027). These significant clinical improvements indicate that ERT with agalsidase alfa may not only halt the progression of Fabry disease, but can also reverse the organ damage resulting from Gb3 storage.

**First evidence that extra-cerebral recombinant Phenylalanine Ammonia Lyase treatment will reduce brain phenylalanine in Phenylketonuria.** C.N. Sarkissian<sup>1</sup>, Z. Shao<sup>2</sup>, M. Pedneault<sup>2</sup>, D.M. Boulais<sup>1</sup>, R. Heft<sup>2,3</sup>, C.R. Scriver<sup>1</sup>. 1) Departments of Biology and Human Genetics, McGill University, Montreal, QC, Canada; 2) IBEX Technologies Inc., Montreal, QC, Canada; 3) BioMarin Pharmaceutical Inc, Novato, CA.

Phenylketonuria (PKU) is a product of deficient (hepatic and renal) phenylalanine hydroxylase enzyme activity, resulting in a metabolic phenotype with elevated levels of phenylalanine (phe) in body tissues and fluids. Persistent increase in extracerebral phe levels is usually associated with elevated intracerebral phe, altered brain structure/function and impaired cognitive development.

PKU responds to treatment with low-phe diet, which lowers ambient phe levels in body fluid. However, compliance with diet is difficult; and for life (as now recommended for some patients), is probably unrealistic. A new potential modality of treatment, involving enzyme substitution with recombinant phenylalanine ammonia lyase (rPAL), converts phe to harmless metabolites (*trans*-cinnamic acid and trace ammonia). Taken orally, non-absorbable and protected PAL effectively lowers plasma phe in mutant hyperphenylalaninemic mouse models (Sarkissian C.N. et al., *PNAS* 96: 2339-2344, 1999).

Here we report the first evidence that extracerebral rPAL treatment reduces brain phe levels. Brain amino acid profiling of PKU mice with hyperphenylalaninemia, following single dose intra-peritoneal treatment with a minimal single dose of 4 IU of non-absorbable formulated rPAL, reveals the anticipated reduction in blood phe levels apparent as of 8 hours (control 179416 M vs rPAL treated 153175 M; p0.024) and a reduction of brain tissue phe concentration at 24 hours post-treatment (control 81741 M vs rPAL treated 71528 M; p0.033). This reduction in whole brain phe is not accounted for by the change in intra-vascular phe reduction. Our findings further support the rationale for rPAL treatment, and are compatible with oral therapy, which favorably alters the flux and equilibrium of phe between the various compartments of body fluids.

**Development of tandem mass spectroscopy for the detection of lysosomal storage diseases from newborn blood spots.** *C.R. Scott<sup>1</sup>, M.H. Gelb<sup>2,3</sup>, F. Turecek<sup>2</sup>, Y. Li<sup>2</sup>, Y. Ogata<sup>2</sup>, D. Wang<sup>2</sup>.* 1) Dept Pediatrics, Univ Washington Sch Medicine, Seattle, WA; 2) Dept Chemistry, Univ Washington, Seattle, WA; 3) Dept Biochemistry, Univ Washington, Seattle, WA.

We are developing tandem mass spectroscopy as a common platform for the detection of lysosomal storage diseases at birth. We have demonstrated that lysosomal enzymes are stable and detectable from blood placed on newborn screening cards. Their enzymatic activity can be measured by sensitive assays that make use of substrate conjugates and Affinity Capture and Elution/Electrospray Ionization Mass Spectrometry (ACESIMS). The method uses a single analytical platform, uses stable isotopes as an internal standard, requires trace amounts of substrates, and is able to be automated for high throughput assays. Selected assays can be multiplexed to measure several enzymes simultaneously.

We have documented that the enzymes responsible for Gaucher, Fabry and Pompe diseases--disorders for which there exists enzyme replacement therapy--can be assayed on dried blood spots. The enzyme activity is linear with respect to time and protein concentration and is stable for several weeks on dried blood spots held at ambient temperature. Additional disorders that are detectable include Krabbe, Niemann-Pick, and selected mucopolysaccharidoses. We believe the method can be developed as a public health approach for the detection of selected lysosomal storage diseases for which there exist specific therapeutic interventions.

Supported by the NIH and Genzyme Corporation.

**The Impact of MPS I on the Functional Status of Affected Individuals.** *A.M. Skrinar, G.F. Cox.* Genzyme Corporation, Cambridge, MA.

**Objective:** Mucopolysaccharidosis 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 resulting accumulation causes clinical manifestations that significantly impair the ability to perform activities of daily living. This study characterized the functional limitations of affected individuals for the purpose of developing a disease-specific functional outcomes measure. **Methods:** A sample of 17 individuals with MPS I participated. Parent proxy responses were solicited for participants under 14 years of age. The sample included 8 males (47%) and 9 females (53%) originating from the U.S., UK, and France. Semi-structured interviews were conducted to collect data on specific limitations in self-care and mobility, as well as caregiver assistance. **Results:** The mean age of participants was 17.7 years (range 7-35). Most had been clinically diagnosed with Hurler-Scheie syndrome (88%). Dressing impairments were reported by 87% of participants and included restrictions in the ability to manage upper and lower body clothing items. Bathing and grooming impairments were reported by 70% of participants and included limitations in the ability to transfer into a bathtub, wash and dry body, and brush hair and teeth. Functional impairments related to feeding were reported by 59% of participants and included difficulty with utensils and the management of food containers. Impaired ability to perform toileting tasks was reported by 47% of participants. Walking device or wheelchair use was reported by 41% of participants. All participants reported some degree of gross motor skill impairment. At least minimal caregiver assistance was required by 88% of participants and none were living independently at the time of the interview. **Conclusions:** Individuals with MPS I presented with heterogeneous functional profiles, although most had significant self-care and motor impairment and required caregiver assistance. These findings provide support for the continued development and validation of a disease-specific instrument to characterize the impact of MPS I on functional status and to assess therapeutic response.

**Clinical response to Enzyme replacement therapy (ERT) with agalsidase beta in a pediatric patient with Fabry disease.** *M.J. Peterschmitt*<sup>1,2</sup>, *P. Della Giovanna*<sup>3</sup>, *N.R. Lago*<sup>4</sup>, *H.A. Repetto*<sup>2</sup>. 1) Neurologia Infantil, Hospital Nacional A. Posadas, El Palomar, Buenos Aires, Argentina; 2) Servicio de Pediatría, Hospital Nacional A. Posadas, El Palomar, Buenos Aires, Argentina; 3) Dermatología, Hospital Nacional A. Posadas, El Palomar, Buenos Aires, Argentina; 4) Catedra de Anatomía Patológica, Universidad de Buenos Aires, Buenos Aires, Argentina.

**Introduction:** In Fabry disease (FD) -an X-linked lysosomal storage disorder- the deficiency of -galactosidase A (-gal A) causes progressive systemic accumulation of glycosphingolipids (GL-3), which leads to the major manifestations of the disease: angiokeratoma, acroparesthesia, renal insufficiency, and cardiovascular and cerebral involvement. ERT with recombinant human -gal A (agalsidase beta) in patients older than 18 years of age has shown improvement of pain and quality of life and clearance of GL-3 deposits in capillary endothelium of kidney, skin and heart. We report the clinical response to agalsidase beta in a pediatric patient with FD. **Case:** An 8 year-old boy presented at 5 years of age with scattered angiokeratoma in his left lower extremity (LLE). Later he developed multiple lesions in his LLE, umbilical region, abdomen and genitalia, teleangiectasia in palms and upper extremities, acroparesthesia, and tortuosity of conjunctival vessels. Decreased -gal A activity confirmed the diagnosis of FD (0.31 nmol/h/ml; NV 2.2-16). Renal function and brain MRI were normal. Ultrastructural examination of renal tissue revealed GL-3 deposits in all cellular types. After approval of Hospital IRB, administration of agalsidase beta (Fabrazyme) was started at 1 mg/kg biweekly. **Results:** The medication was well tolerated. After 2 months on treatment, the extremity pain ceased and the skin lesions began to disappear. **Conclusion:** Our patient showed regression of some of his angiokeratoma, which has not been reported in enzyme-treated FD. Treatment started at an earlier stage of the disease could yield greater benefit. Long-term safety and effectiveness of ERT in the pediatric population should be evaluated in randomized clinical trials.

**Discovery of new members of the acyl-CoA dehydrogenase (ACD) gene family.** *M. He, W. Moshen, J. Vockley.*  
Dept of Medical Genetics, Mayo Clinic, Rochester, MN.

The ACDs are a family of flavoenzymes consisting of 9 distinct members involved in fatty acid -oxidation and amino acid catabolism. Using a bioinformatics approach, we have identified a new group of ACD-like enzymes (ACDXs) that are 20-30% homologous to the ACDs. Two of them, ACDX1 and ACDX2, are 80% homologous between human and mouse, while human ACDX1 is 46% homologous to ACDX2. The human ACDX1 gene is located on chromosome 12 and EST database searches reveal 7 distinct messages from this gene apparently generated by differential splicing. One encodes an ACD-like protein, while the others lead to ACD-like proteins with an aminoglycoside phosphotransferase domain at the N-terminus, an aldehyde dehydrogenase-like sequences at the C-terminus, or both. A second human gene, ACDX2, is located on chromosome 3. EST database searches identify four different apparent RNA species, two ACD-like proteins with N termini of variable length, and two with an aminoglycoside phosphotransferase domain at the N terminus. Computer modeling of ACDX2 based on the structures of known ACDs predicts the catalytic base of ACDX2 to be an Asp instead of Glu as seen in other ACDs. Other residues around the substrate binding pocket are well conserved. An Arg residue located in the substrate binding pocket of ACDX2 is near the catalytic base, in a position homologous to Arg94 in glutaryl-CoA dehydrogenase (GAD). Arg94 is unique to GAD and this suggests that ACDX2 might react with a similar hydrophilic substrate and perform a decarboxylation reaction as does GAD. We have amplified the ACD-like domain of ACDX2 from liver mRNA. In vitro transcription and translation generated two stable proteins, one consistent with translation of the full message (511aa, 57KDa), the other with initiation from an internal Met codon and generating a shorter protein (464aa, 52KD). Only the 52KD protein was imported into isolated liver mitochondria in an in vitro assay. Thus the alternative N-termini of the ACDXs may serve to target them to different subcellular locations as well as confer additional enzymatic functions. Further functional characterization of this new group of ACDs is underway.

**Spectrum of GC gene mutation among Korean Gaucher disease patients and its implications on genotype and phenotype correlation.** *H. Kim, J.A. Yang, S.J. Park.* Dept Medical Genetics, Ajou Univ Col Medicine, Suwon, Korea.

Gaucher Disease(GD) is the most prevalent lysosomal storage disease due to deficient activity of Glucocerebrosidase (GC). The clinical study of Korean GD registry revealed 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 among Korean GD pts (>50%). To understand the molecular basis of these phenotypic characteristics of Korean GD, mutational analysis of GC gene was performed in 25 unrelated Korean GD pts (12 type1, 6 type2, 4 type3A, 3 type3B) and 15 different mutations were identified in 50 alleles; 10 alleles for L444P mutation, 7 alleles for G46E, 4 alleles each for F213I, R257Q and N188S. 3 alleles for G202R, and 9 rare mutations of R48W, L160V, V191G, A232P, F331L, V375V, G377S, D409H and V460V an allele for each. The spectrum of GC mutations found in Korean GD is rather unique compare to those of Jewish & Caucasian population. N370S, the most common GC mutation among Jewish (70%) and other Caucasian ethnic group (60-30%), and well established for its association with mild non-NP and the protective mutation from NP, is not found in Korean. and Thus, the lack of N370S mutation among Korean may explain type 1 being severe 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 homo alleles were detected. Exception of G46E, among next common mutations of F213I, R257Q and N188S were also detected in both type I and NP pts. An interesting finding was G46E mutation, never reported in Caucasian nor Japanese GD, was found to be the most common alleles occurring 50% of type I pts. but none in NP pts. Therefore finding of G46E allele may provide a predictive value for non-NP phenotype esp. DDX in type1 vs. type3B among early onset Korean GD pts.

**Relationship between metabolites, growth and galactose exposure in yeast deficient in the Leloir pathway enzymes.** *K.L. Ross, J.L. Fridovich-Keil.* Human Genetics, Emory University, Atlanta, GA.

The metabolism of galactose via the three enzymes of the Leloir pathway: galactokinase (GALK, *GAL1*), galactose-1-P uridylyltransferase (GALT, *GAL7*), and UDP galactose-4-epimerase (GALE, *GAL10*) is a process that has been conserved from *E. coli* through humans. Impairment of this pathway in humans results in the disorder galactosemia, with precise symptoms and severity a function of which enzyme is impaired, and the degree of impairment. Of the three enzyme deficiencies, transferase-deficiency galactosemia, also known as classic galactosemia, is the most common and clinically significant. Dietary restriction of galactose remains the only known treatment, and despite early detection and intervention, a large fraction of patients experience serious clinical complications. The underlying pathophysiology in galactosemia remains unknown, limiting possibilities for the generation of novel and potentially more effective therapies.

As an approach to understand the biochemical basis of galactosemia, we have begun to explore the functional and metabolic implications of impaired galactose metabolism in yeast. In brief, we have generated strains deficient in *gal1*, *gal7*, and *gal10*, and have systematically tested both growth rates and relevant metabolic profiles for these cells cultured under conditions of exposure to different levels of galactose. Thus far it appears that *gal10* deficient yeast growth arrest at a 10-fold lower concentration of external galactose than do *gal7* deficient yeast. In addition, within 5 min of exposure to galactose there are clear differences in the internal galactose metabolites, specifically galactose-1-phosphate, between wild-type cells and those deficient in *gal7* or *gal10*. Our results clearly demonstrate reproducible changes in both growth and metabolites in these cells in response to galactose, and set the stage for future experiments designed to test the relationship between genetic background, specific Leloir enzyme activities, accumulation and/or depletion of specific metabolites, and galactose-induced growth arrest in yeast.

**Immunological aspects of X-linked ectodermal dysplasia: A dog model for the human disease.** *M. Casal<sup>1</sup>, S. Ryan<sup>2</sup>, J. Rhodes<sup>2</sup>, J. Scheidt<sup>1</sup>*. 1) Dept Medical Genetics, Univ Pennsylvania, Phila., PA; 2) Veterinary School, Univ Pennsylvania, Phila., PA.

As in most human patients with X-linked ectodermal dysplasia (HED), our HED dogs are at an increased risk for pulmonary disorders. Localized immune system defects had been suspected previously in the ectodermal dysplastic dogs because of the frequent infections and unexpected deaths due to opportunistic respiratory tract infections. Experiments were designed to examine systemic and localized (pulmonary) humoral and cellular responses, development and function of T cells and thymic morphology. All dogs (5 normal and 5 affected dogs) used in these experiments were clinically healthy at the time of examination and their immune responses were compared to normal littermates. Serum immunoglobulin concentrations did not differ between normal dogs and dogs affected with HED, and the HED dogs responded appropriately to vaccination with tetanus toxoid suggesting normal systemic B and plasma cell function. Quantitative examination of B cells from blood and lymphoid organs was also within normal limits except in blood from carrier dogs (heterozygous females) when compared to normal dogs. Total CD3 and the percentage of CD8 cells were lower in affected dogs than those in normal dogs. However, all other thymic parameters were within normal limits suggesting normal development of T cells. Thymic morphology was compared between normal and affected dogs and T cells were assessed for functionality. There were no significant differences between normal and affected dogs. Macrophages and neutrophils were examined for cytotoxic and phagocytic ability as well as cytokine production. There was no significant difference between normal and affected dogs. In contrast, the secretory IgA concentrations found in affected dogs were significantly higher than in normal dogs, but secretions such as lachrymal fluids were significantly decreased. These results suggest a compensatory mechanism for secretory IgA, so that the total amount equals that in normal dogs. The results indicate that the HED dogs have a relatively intact immune system.

**Molecular characterization of disease-causing mutations in six patients with GM1 gangliosidosis.** A. Caciotti<sup>1</sup>, T. Bardelli<sup>1</sup>, P. Scarnato<sup>1</sup>, M. Taverna<sup>1</sup>, M.A. Donati<sup>1</sup>, V. Benigno<sup>2</sup>, A. Boneh<sup>3</sup>, V. Kimonis<sup>4</sup>, A. d'Azzo<sup>5</sup>, E. Zammarchi<sup>1</sup>, A. Morrone<sup>1</sup>. 1) Dept of Pediatrics, Florence, Italy; 2) Dept of Pediatrics, Palermo, Italy; 3) Metabolic Service, Gen Health Serv, Victoria, Australia; 4) Children's Hosp Harvard Med School, Boston, USA; 5) Dept of Genetics, St. Jude Hosp, Memphis, USA.

GM1-gangliosidosis is a lysosomal storage disorder caused by a deficiency of beta-galactosidase (GLB1). The severe type I infantile form is characterised by a rapidly progressive CNS involvement, facial and skeletal abnormalities and hepatosplenomegaly. The GLB1 gene gives rise to two alternately spliced mRNAs: the GLB1 lysosomal enzyme and the Elastin Binding Protein (EBP), a major component of the non-integrin cell surface receptor complex. We report the identification of 5 new GLB1 mutations (amino acid substitutions H281Y, T239M, Q355H; c1309delA and IVS6+2TC splicing defect) and 3 known (R482H, R59C and R208C) mutations in the total RNA and DNA preparation of six GM1 Gangliosidosis patients (two American, two Australian, and two Italian). Except for the R208C and the T239M mutations, all genetic lesions detected affect both the GLB1 and EBP proteins. Hot spot regions for the GLB1 mutations have been suggested and both T239M and R208C, mapping to exon 6, confirm this exon to be a hot spot. The R482H amino acid substitution has been previously reported at a homozygous level in one Italian patient and at a heterozygous level in 6 unrelated Italian patients, all of Sicilian origin. In the current work, the R482H mutation was identified in two Australian patients of Maltese origin. Thus, it can be surmised that this mutation has a Mediterranean origin. The R208H mutation has been reported to be of Hispanic origin, although it was found at high frequency in American patients. The involvement of the R59 amino acid has been previously reported in patients of Italian and Brazilian origin. Sequence alignments of GLB1 related proteins, indicated that the R59 amino acid should have a key role. These data could reflect the migratory flux from Europe to America and Australia. The financial support of AMMEC and MPS ONLUS is gratefully acknowledged.

**Neuropathological analysis of Sandhoff disease mouse model.** *C. Bourgoin*<sup>1</sup>, *A. Gelot*<sup>2</sup>, *R. Gravel*<sup>3</sup>, *L. Poenaru*<sup>1</sup>, *C. Caillaud*<sup>1</sup>. 1) Department of Genetics, Institut Cochin, Paris, France; 2) Neuropathology Unit, Trousseau Hospital, Paris, France; 3) Department of Cell Biology, University of Calgary, Calgary, Canada.

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 beta-chain. A *Hexb*<sup>-/-</sup> knock-out mouse model has been developed. It displays a phenotype close to the human pathology, leading to death at the age of 4 months. Regional and cellular lesions were studied in *Hexb*<sup>-/-</sup> mice brain, as well as cellular storage at different time points. Cellular parameters were cytoplasm distension, vacuolisation and lipid positive staining. Cell counting allowed to assess cell loss, as well as the percentage of neurons overloaded for each region. The main conclusions of the study were: (i) Each cerebral structure studied displays a different chronology in the storage, the earliest overloaded regions being the central grey nuclei of the thalamus, and the Purkinje cells. (ii) A schematic sequence of events was deduced: neurons first display a lipid positive overload, without distension of the cytoplasm. The overload becomes then more diffuse, with a swelling of the cytoplasm, that undergoes vacuolisation and loses lipid staining. (iii) The storage does not lead to early and extensive cell loss, indicating that a cell dysfunction rather than massive cell loss would trigger clinical signs. The clear differential between deep and superficial layers in highly structured regions, as well as between metabolically different cells indicates a role of synaptogenesis in the storage process, likely due to the high membrane turn-over in these cells. In addition to the better understanding of the pathological process, the kinetic data of the storage apparition will allow to evaluate the efficacy of preclinical gene transfer into the brain, in terms of neuron morphology and storage.

**Fabry disease : a comprehensive study of renal function in 28 hemizygous male patients.** *D.P. Germain<sup>1</sup>, D. Erlich<sup>2</sup>, S. Sainte-Croix<sup>1</sup>, T. Le Bricon<sup>2</sup>, Y. Herrera-Guzman<sup>1</sup>, A. Bissery<sup>3</sup>, C. d'Auzac<sup>4</sup>, P.F. Plouin<sup>5</sup>, M. Froissart<sup>6</sup>.* 1) Dept of Genetics, European Hosp Georges Pompidou, Paris, France; 2) Dept of Biochemistry, St Louis Hosp, Paris, France; 3) CIC, European Hosp Georges Pompidou, Paris, France; 4) Dept of Nephrology, European Hosp Georges Pompidou, Paris, France; 5) Dept of Medicine, European Hosp Georges Pompidou, Paris, France; 6) Dept of Physiology, European Hosp Georges Pompidou, Paris, France.

**Background:** Fabry disease (FD) is an X-linked inborn error of metabolism due to the deficient activity of alpha-galactosidase A, leading to the lysosomal accumulation of glycosphingolipids. **Objective:** To characterise renal function in male patients affected with classic FD and to evaluate cystatin C, a new marker of renal function. **Methods:** Serum creatinine, measured creatinine clearance using 24h-urine collection, cystatin C, glomerular filtration rate (GFR) measured by chrome 51 EDTA and 24h proteinuria (24h/P) were performed in 28 consecutive hemizygous men (mean age 32 y, 16-66), excluding patients on dialysis or with kidney transplant. **Results:** Mean Cr51 EDTA GFR (n=20) was 79.5 mL/min (21-114 mL/min). 9 patients had normal kidney function (GFR90mL/min), 6 had mild kidney failure (60-89 mL/min), 3 had moderate kidney failure (30-59 mL/min), and 2 had severe kidney failure (GFR30 mL/min). Mean cystatin C (n=23) was 1.06 ng/L (Normal: 0.50-0.94 ng/L). Mean serum creatinin (n=28) was 106 mol/L (Normal: 50-115 mol/L). For the subset of patients (n=16) for which both GFR and cystatin C were studied, there was a remarkable correlation. Eight patients had no proteinuria, 12 had microalbuminuria (30-300 mg/24h), 5 had non-nephrotic 24h/P (300mg-3g/24h) and 3 had 24h/P >3g/24h. **Discussion:** Kidney failure was frequent in patients with FD. The high prevalence of proteinuria (71%) suggests the potential benefit of angiotensin converting enzyme inhibitors for nephroprotection. The high sensibility of cystatin C for kidney failure (87.5%) was demonstrated by measurement of the gold standard GFR. Evaluation of FD patients should include cystatin C at baseline for easy monitoring of the efficacy of enzyme replacement therapy on kidney function.

**Mucopolysaccharidosis VI: clinical, biochemical and molecular analysis of 28 South American patients. R.**

*Giugliani<sup>1,4,5</sup>, A.C. Azevedo<sup>1,6</sup>, M. Petry<sup>1,7</sup>, A.P. Behregaray<sup>1</sup>, F.T. Souza<sup>1</sup>, M.B. Rosa<sup>1</sup>, C. Giugliani<sup>1</sup>, S. Brustolin<sup>1,6</sup>, L. Kalakun<sup>1</sup>, S. Canani<sup>2</sup>, D. Marinho<sup>3</sup>, P. Esteves<sup>3</sup>, T. Dieter<sup>1</sup>, M. Burin<sup>1,7</sup>, S. Leistner-Segal<sup>1,6</sup>, I.V. Schwartz<sup>1,5</sup>.* 1) Med Genet Serv and; 2) Pneum Serv and; 3) Ophthalm Serv of Hosp Clin P Alegre; 4) Dep Genet and; 5) Postgrad Course Genet Molec Biol and; 6) Postgrad Course Pediat and; 7) Postgrad Course Biochem, UFRGS, Porto Alegre, RS, Brazil.

Mucopolysaccharidosis VI (MPS VI) is a lysosomal disease caused by the deficiency of the enzyme N-acetylgalactosamine-4-sulfatase (ARSB). To perform a comprehensive evaluation of a cohort of South American MPS VI subjects, 28 patients with a clinical and biochemical diagnosis of MPS VI (1/3 of the total number diagnosed at our center), with no history of treatment through BMT and/or ERT, were evaluated by means of anamnesis, physical examination, visual acuity and intra-ocular pressure measurements, biomicroscopy, echocardiogram (ECHO), electrocardiogram (EKG), spirometry, 6-minute walking test (6MWT), polysomnography (PSG), and analysis of ARSB gene. Mean age at evaluation was 97.1 mo.; 25/28 patients reported onset of symptoms before the age of 36 months; mean age at diagnosis was 48.4 mo. ARSB activity was deficient in all patients (means of 5.4 nm/h/mg prot. in leucocytes and 38.4 nm/h/mg prot. in fibroblasts). All patients presented with increased urinary excretion of GAGs (3 times increase in average). Of the 51 signs/symptoms surveyed through anamnesis and physical examination, short stature, coarse facial features, corneal opacification, joint stiffness, and claw hands were reported in all cases. The molecular analysis identified the presence of a new mutation in 6/50 alleles, all of these from the Southeast region of Brazil. Besides providing a better understanding of the natural history of MPS VI, our data confirm that high morbidity-mortality is associated to this disease, and that the identification/treatment of its complications usually takes place in a later stage. The identification of a new mutation in patients may be a clue to investigate the apparently high frequency of MPS VI in Brazil, particularly on the Southeast region (CAPES/CNPq/Biomarin).

**Natural History of Infantile Onset Pompe Disease(IOPD): A Study Report.** *D. Corzo*<sup>1</sup>, *W.L. Hwu*<sup>2</sup>, *H. Mandel*<sup>3</sup>, *M. Nicolino*<sup>4</sup>, *F. Yong*<sup>1</sup>, *P. Kishnani*<sup>5</sup>. 1) Genzyme Corporation, Cambridge, MA; 2) National Taiwan University Hospital, Taiwan, Taipei; 3) Rambam Medical Center, Haifa, Israel; 4) Hopital de Brousse, Lyon, France; 5) Duke University Medical Center, Durham, NC.

**Background.** Reports on the natural history of IOPD are based on data from small number of patients. To fully characterize it, a retrospective chart review was conducted. **Methods.** This was a multinational, multicenter, historical cohort study. Inclusion required documented onset of symptoms by 12 months of age and GAA enzyme deficiency or GAA mutation(s). Data collected included demographics, family history, progression of signs and symptoms by organ system, diagnostic process, ancillary evaluations, treatment modalities, and resource utilization. **Results.** 300 cases were screened; 168 cases from 33 study sites in 9 countries met all eligibility criteria. 93 cases (55%) were born during/after 1995 and 75 cases (45%) were born before 1995. Median age  $\pm$  SD (months) at presentation of first symptoms was  $2.0 \pm 2.5$  (n=166); at diagnosis,  $4.7 \pm 8.8$  (n=165); at first ventilator use,  $5.9 \pm 6.3$  (n=165); and at death,  $8.7 \pm 1.1$  (n=163). By 12 months of age mortality was 78%; by age 18 months mortality reached 88%. The most common signs/symptoms were cardiomegaly (92%, n=154), hypotonia (88%, n=148), cardiomyopathy (88%, n=147), respiratory distress (78%, n=131), failure to thrive (53%, n=89), congestive heart failure (50%, n= 84), and pneumonia (45%, n=76). At least 29% of patients (n=49) were ventilated. 90% (n=151) were treated with  $\geq 1$  medication(s), 77% (n=130) with nutritional support, and 93 (55%) with other supportive therapies. **Conclusions.** This is the largest retrospective case review study performed to date in IOPD. Results from this study support and extend earlier literature reports with regard to the fatal course of the disease and the rapidity of the disease progression. In spite of widespread use of medications and other therapeutic modalities, mortality has changed little across the decades. Although a small group of patients have a longer survival, IOPD is still a rapidly progressive disease, with the majority of patients dying before 12 months of age.

**New mutations in the PPCA gene lead to the alteration of the Lysosomal complex and the EBP-receptor. S.**

*Malvagia<sup>1</sup>, A. Morrone<sup>1</sup>, A. Caciotti<sup>1</sup>, T. Bardelli<sup>1</sup>, A. d'Azzo<sup>2</sup>, G. Ancora<sup>3</sup>, M.A. Donati<sup>1</sup>, E. Zammarchi<sup>1</sup>.* 1) Dept of Pediatrics, Univ. of Florence, Azienda Ospedaliera Universitaria Meyer, Florence, Italy; 2) Genetics, St. Jude Children's Res Hospital, Memphis, TN, USA; 3) Inst of Neonatology, University of Bologna, Italy.

We describe the clinical findings, the biochemical and molecular analyses of an Italian family in which recurrent hydrops fetalis due to Galactosialidosis (GS) occurred. GS is a very rare Lysosomal Storage Disorder caused by a deficiency of the protective protein/cathepsin A (PPCA). This protein forms a high-molecular-weight multicomplex with the hydrolases galactosidase (GLB1) and neuraminidase (NEU1), defending them against rapid proteolytic degradation and allowing them to take on the correct folding. Controversial data shows that PPCA is also present in a second complex, the EBP-receptor, involved in elastogenesis with Elastin Binding Protein (EBP) and NEU1. We investigated the potential role of the PPCA in both complexes. Two new genetic lesions (c60delG and IVS2+1 GT) that lead to a frameshift and a premature stop codon were detected in the patients PPCA cDNA and genomic DNA. The deleterious effect of such mutations was also confirmed by the absence of PPCA with western blot analysis. Since no PPCA protein was detected, analysis of the patients fibroblasts can be considered an effective means of examining the role and effect of PPCA on the two complexes. Interestingly, a reduced amount of both GLB1 and EBP proteins in the patient's fibroblasts was also detected. These data confirm that PPCA is present in two functional complexes, inside lysosomes with GLB1 and on the cell surface with EBP. The reduction in GLB1 and EBP confirms that PPCA is essential for their integrity. We would like to alert physicians to consider an inborn error of metabolism in patients with hydrops fetalis where an immunological cause has been excluded, and with a positive family history. The financial support of AMMEC, MPS Onlus and Az. Osp. Meyer are gratefully acknowledged.

**Substrate specificity and multiple tissue isoforms of glucocerebrosidase in patients with Gaucher disease. E.**

*Orvisky<sup>1, 2</sup>, B.M. Martin<sup>3</sup>, J.M. Walker<sup>1, 2</sup>, E. Sidransky<sup>1, 2</sup>.* 1) Section on Molecular Neurogenetics NIH/NIMH, Bethesda, MD; 2) Medical Genetics Branch, NIH/NHGRI, Bethesda, MD; 3) Laboratory of Neurotoxicology, NIH/NIMH, Bethesda, MD.

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. Multiple isoforms of GC have been demonstrated in fibroblasts with molecular weights of 63, 61 and 59 kDa, where the 59 kDa form is believed to be the functional GC. We have previously shown that isoforms of GC from brain differ from those from other tissues. Western blots performed on protein extracts from normal brains consistently showed two GC bands, one at 56kDa and one at 59kDa. After N-Glycosidase F digestion, all samples demonstrated only the lowest 56kDa form of glucocerebrosidase regardless of the tissue source. 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. Brain samples from patients with all three types of Gaucher disease predominantly had the 56kDa form, but all were deficient in the functional 59kDa form. We were unable to distinguish the neuronopathic from the non-neuronopathic form of Gaucher disease based on either residual GC activity or GC isoforms. However, residual GC activity was markedly reduced by competitive inhibition by the alternate substrate glucosylsphingosine in fibroblasts and tissue extracts from patients with the neuronopathic form of GD but not in patients with type 1 GD. This substantiates the role of glucosylsphingosine in the pathogenesis of Gaucher disease.

**Localization and clathrin binding of the Hermansky Pudlak syndrome type 3 protein.** A. Helip Wooley, H. Dorward, W.A. Gahl, M. Huizing. National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Hermansky Pudlak syndrome (HPS) is a disorder of lysosome-related organelle biogenesis resulting in melanosome dysfunction and absent platelet dense bodies. HPS is characterized clinically by oculocutaneous albinism and prolonged bleeding times due to a platelet storage pool deficiency. HPS-3 is a relatively mild subtype of HPS and results from mutations in the *HPS3* gene located on 3q24. *HPS3* encodes a 1004 amino acid protein of unknown function with no apparent homology to other human proteins or protein domains. HPS3 contains a clathrin binding motif (LLDFE) at residues 172-176 and a potential ER retention signal (KKPL) at residues 1000-1003. To determine if HPS3 binds clathrin, an HPS3-GFP fusion construct was created and site-directed mutagenesis was used to alter the HPS3 clathrin binding motif to non-conserved amino acids, i.e., AAAPG. Wild-type and mutant HPS3-GFP were expressed in normal human fibroblasts, immunoprecipitated with GFP antibodies, and analyzed by western blot using clathrin heavy chain (CLH) antibodies. A band corresponding to CLH (MW 187 kD) was detected in wild-type HPS3-GFP but not mutant HPS3-GFP immunoprecipitates. Normal human melanocyte lysates were immunoprecipitated with either CLH or HPS3 peptide antibodies and western blots performed using the alternate antibody. CLH was immunoprecipitated with HPS3 and vice versa. Fluorescence imaging of HPS3-GFP expressed in human melanocytes demonstrated localization to the perinuclear region as well as punctate structures in the dendrite tips. Immunofluorescence using HPS3 peptide antibodies in untransfected melanocytes confirmed this localization. These studies demonstrate that HPS3 binds clathrin and provide insight into the function of HPS3 and its role in intracellular vesicle trafficking.

**Apoptosis in brain in the type 2 Gaucher mouse: neurotoxicity starts *in utero*.** M.E. LaMarca<sup>1</sup>, O. Goker-Alpan<sup>1</sup>, E. Orvisky<sup>1</sup>, E.I. Ginns<sup>2</sup>, E. Sidransky<sup>1</sup>. 1) Molecular Neurogenetics Section, NIMH and Molecular Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Brudnick Neuropsychiatric Research Institute, U. Mass. Medical School, Worcester, MA.

Gaucher disease, the inherited deficiency of glucocerebrosidase, presents with both non-neuronopathic and neuronopathic phenotypes. Despite many years of research, the mechanisms resulting in neuronopathic Gaucher disease are not fully understood. The presence of perivascular Gaucher cells, neuronophagia and gliosis as the primary neuropathological findings suggest that a toxic metabolite might cause the neuronal dysfunction. Although both glucosylceramide (Glc-cer) and glucosylsphingosine (Glc-sph) accumulate in visceral organs of patients with all types of Gaucher disease, elevated levels in brain occur exclusively in neuronopathic patients. Similarly, in the null allele Gaucher mouse, a model for type 2 Gaucher disease, Glc-sph levels are elevated approximately 100-fold when compared to Glc-sph levels in wild-type littermates. As previously shown, Glc-sph can be detected in homozygous null allele mice by gestational age E-13 and progressively accumulates until birth. Ceramide has been implicated to have a central role in the signaling mechanisms of apoptosis, while both Glc-cer and Glc-sph are associated with growth arrest. To determine the potential neurotoxicity of substrate accumulation, we performed a DNA fragmentation (TUNEL) assay on brain samples from E-19 mouse embryos. A significantly increased number of apoptotic cells were detected in homozygous null allele embryos as compared to heterozygous or normal littermates. Some were localized in bands extending from mid to hindbrain. The presence of apoptotic cell clusters in these embryos in patterns similar to the neuropathology observed in some type 2 patients provides further evidence that substrate toxicity beginning *in-utero* results in irreversible neuronal dysfunction and subsequent death.

**Hermansky-Pudlak syndrome type 5 and type 6: Four new patients.** *R.A. Hess<sup>1</sup>, D.A. Claassen<sup>1</sup>, J. White<sup>2</sup>, W.A. Gahl<sup>1</sup>, M. Huizing<sup>1</sup>.* 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) University of Minnesota, Minneapolis, MN.

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 granulomatous colitis or pulmonary fibrosis. Six genes are now associated with HPS in humans, and there are at least 14 mouse models with the HPS phenotype. Two of the human genes are *HPS5* and *HPS6*, the homologues of the murine *ruby eye-2* and *ruby-eye* loci, respectively. *HPS5*, on 11p14, consists of 23 exons (coding for 1129 aa) and is expressed as at least two alternatively spliced mRNA transcripts differing at the 5' ends. Human *HPS6*, located on 10q24.32, consists of 2 exons coding for a 775 aa protein.

HPS-5 and HPS-6 have been described in only one patient each. We studied 15 patients whose HPS-causing gene was unknown, to identify other individuals with HPS-5 or HPS-6. Using PCR amplification of cDNA and gDNA followed by direct sequencing, we identified 2 new HPS-5 patients bearing a total of 4 different mutations, including 3 frameshift (P239insC, L875delT, R216delAGAT), and one nonsense (R865X). Northern blots showed severely decreased *HPS5* mRNA levels in both patients, attributable to nonsense mediated RNA decay. Molecular analyses also revealed two HPS-6 patients, each carrying a homozygous mutation Q305X and 622delTG. The possibility of hemizygoty will be examined.

Clinically, our HPS-5 and HPS-6 patients exhibited iris transillumination, variable hair and skin pigmentation, and absent platelet dense bodies. Pulmonary fibrosis and granulomatous colitis were not observed in these patients. However, all of the patients were under 27 years old, an age before which lung disease rarely develops in any type of HPS. Hence, it is important that adults with HPS-5 and HPS-6 continue to be followed for the development of restrictive lung disease. The diagnosis of a particular subtype of HPS, such as HPS-5 or HPS-6, might influence future treatment decisions; a clinical trial has recently shown that the antifibrotic agent, pirfenidone, slows the decline in pulmonary function associated with HPS-1 disease.

**Clinical, biochemical and molecular diagnosis of a free sialic storage disease patient of moderate severity. R.**

*Kleta<sup>1</sup>, R.P. Morse<sup>2</sup>, J. Alroy<sup>3</sup>, E. Orvisky<sup>4</sup>, D. Krasnewich<sup>1</sup>, D.A. Wenger<sup>5</sup>, W.A. Gahl<sup>1</sup>.* 1) Section on Human Biochemical Genetics, MGB, NHGRI, NIH, Bethesda, MD; 2) Pediatrics, Dartmouth-Hitchcock Medical Center, Lebanon, NH; 3) New England Medical Center, Boston, MA; 4) NSB, NIMH, NIH, Bethesda, MD; 5) Neurology, Jefferson Medical College, Philadelphia, PA.

The diagnosis of patients with developmental delay in early childhood is difficult and challenging. The allelic lysosomal storage disorders Salla disease and infantile free sialic acid storage disease (ISSD) should be included in the differential. ISSD has a severe phenotype with infantile onset; Salla disease has a milder phenotype with later onset. ISSD and Salla disease result from mutations in SLC17A5, which codes for sialin, a lysosomal membrane protein that transports the charged sugar, N-acetylneuraminic acid (sialic acid (SA)), out of lysosomes. We describe a 26-month old Caucasian (non-Finnish) child with global developmental delay of postnatal onset, hypotonia, mild but progressive coarsening of facial features, and language and motor skills stagnant at a 3-4 months developmental level. Urinary excretion of free sialic acid was elevated. EM of a skin biopsy revealed enlarged secondary lysosomes consistent with oligosaccharide storage. Free SA in fibroblasts was 3.7+/-1.1 nmol/mg protein (normal 1.1+/-1.0); differential centrifugation indicated a lysosomal location. Genomic analysis revealed compound heterozygosity for two new sialin mutations. In exon 2, a heterozygote 291 G>A, "exonic" donor (5') splice site mutation IVS 2-1 leads to skipping of exon 2; cDNA analysis showed a 197-bp deletion. The exon 2 skipping causes termination after 33 amino acids instead of 495. A second mutation in exon 9, 1226 G>A, GGA>GAA, G409E, changes a conserved glycine to glutamate within a transmembrane region. Each mutation was present in one parent. This child's clinical manifestations of a lysosomal free SA storage disease correlate with her sialin mutations and biochemical findings, elevated fibroblast free SA. Thus, in the proper clinical setting, differential diagnosis of postnatal developmental delay should include free SA storage disorders such as ISSD and Salla disease.

**Demography of untreated Type 1 Gaucher disease (GD1) in children.** *P. Kaplan, H.C. Andersson, J. Charrow, A. Prakash-Cheng, E.H. Kolodny, P. Mistry, G.M. Pastores, B. Rosenbloom, C.R. Scott, R.S. Wappner, N. Weinreb.* ICGG Registry, Cambridge, MA.

Data from the International Collaborative Gaucher Group Registry were analyzed to determine childhood manifestation, ethnic distribution, genotypes, baseline spleen, liver, hematologic indices, bone disease & growth. Data were available for 1578 subjects diagnosed <18yrs. In GD1, 66% of all patients are symptomatic by 18 yrs, of whom 53% manifest in the first 5 yrs, 24% from 6-10 yrs & 23% from 11-18 yrs. Male/ female ratio was 47/53 (739/839). Rare/unique genotypes were most prevalent, affected many ethnic groups & were associated with severe disease. N370S/N370S, most common in adults, accounted for only 8% of children <5 yrs, 22% from 6-10 yrs, and 34% from 11-18 yrs. Linear growth: median z scores <5yrs were 1.31 [n=132], 6-10yrs were 1.32 [n=44] & 11-18yrs were -0.94[n=48]. z score -1.56 is equivalent to 5% ile. Baseline median spleen volumes were higher in children with onset in the 1st decade [18.47 multiples of normal (MN) <5yrs, 13.8 MN 6-10yrs] than in the 2nd decade [ MN 9.01 11-18yrs]. Median hepatomegaly was greater in the 1st decade [2.5 MN <5yrs, 1.8MN 6-10yrs] than 2nd [1.48MN 11-18yrs]. Platelet & hemoglobin [Hb] levels were not meaningfully different across the 3 age categories [115K,120K, 99K; 10.3,10.9,11.7gm% respectively]. Splenectomies were done in 7.6% manifesting children. Bone signs/symptoms (pain/crises/osteoporosis /marrow infiltration/necrosis) were less frequent in children manifesting in the 1st decade than the 2nd decade, although due to small numbers of patients with available data only avascular necrosis showed a statistically significant difference being higher in the 2nd decade. Conclusions: GD1 has a high prevalence in childhood, with unusual/rare genotypes linked to manifestation of disease at an earlier age, without predilection for ethnic groups. Somatic and growth manifestations are severe, and are worse in the 1st decade. Bone disease is less frequent in 1st decade than 2nd. It is possible that the introduction of enzyme replacement therapy in 1991 obviated the need for splenectomies.

**Early Presentation of Fucosidosis.** *F.J Stewart<sup>1</sup>, A.C. Magee<sup>1</sup>, C. Lundy<sup>2</sup>*. 1) Dept Medical Genetics, Level A, Belfast City Hosp, Belfast, United Kingdom; 2) Royal Belfast Hospital for Sick Children, Belfast, United Kingdom.

Fucosidosis is a rare autosomal recessive inborn error of metabolism caused by a deficiency in alpha-fucosidase. Many reports tend to describe older individuals who have a characteristic pattern of angiokeratomata on their hands and feet. The proband is the first child of consanguineous parents who are first cousins. She was born at term weighing 2.27kg. It had been noted that there was poor fetal growth towards the end of the pregnancy. As a baby she had a continual nasal discharge and frequent upper respiratory infections. She did not sit unsupported until the age of 14 months and did not walk until the age of 2 years. She was seen in the genetics clinic at the age of 2 years. On clinical examination she had a very round face with a flat broad nasal bridge. The nose was short and slightly widened. She had macroglossia and a tendency to protrude the tongue. Because of the history of chestiness urine was sent for mucopolysaccharide screening and blood for lysosomal enzymes. The result showed an alpha-fucosidase level of 0.4 umol/g/h (normal 50-200) giving a diagnosis of fucosidosis. Recent review at the age of 4 years has shown her to have marked developmental delay. However she is making slow progress and has not lost any skills. She makes some noises but has no proper speech. There is no progress on toilet training. Her physical appearance has changed somewhat and is now more typical of a child with a storage disorder. She has developed multiple angiokeratomata on her legs and her hands. Fucosidosis is a very rare condition and we know of no other cases in Ireland. The condition is often divided into a severe infantile form, type I, with death before the age of 5 and type II which is said to be due to a partial deficiency where survival into adulthood is common. This girl has a very marked enzyme deficiency but is in good health and likely to survive for several more years. We present this case to draw attention to this disorder and to illustrate that screening urine alone will not detect all lysosomal enzyme deficiencies. At the time of writing results of mutation analysis are not available.

**Dissection of the protective domain of saposin C that is required for resistance of acid -glucosidase to proteolytic degradation.** *Y. Sun, X. Qi, G.A. Grabowski.* Dept Human Genetics, Children's Hosp Research Fndn, Cincinnati, OH.

Saposin C is one of the four small sphingolipid activator proteins that are derived by proteolytic processing of a common precursor, prosaposin. Saposin C is essential for optimal *in vitro* and *in vivo* hydrolysis of glucocerebroside by acid -glucosidase (GCCase), the Gaucher disease enzyme. We have shown that saposin C also is required for GCCase resistance to proteolytic degradation, a new property for saposin C. An activation and protection domain map of saposin C was developed by using a series of saposin C peptides and monitoring their effects on GCCase activity in intact prosaposin deficient cells. A chimeric saposin, CB, contained the NH<sub>2</sub>- and COOH- terminal halves of saposin C and B, respectively. CB produced an increase of 2-fold in GCCase activity similar to saposin C. A saposin C with mutations in the activation domain similarly enhanced GCCase activity. However, saposin BC, containing the NH<sub>2</sub>- and COOH-terminal halves of B and C, respectively, had little effect on GCCase activity. These results show that the activation domain is separated from protective domain on saposin C. The amino terminal half of saposin C is important for GCCase proteolytic protective functions. Thus, saposin C appears to be a multifunctional molecule (protection and activation) in maintaining normal GCCase function *in vivo*. These functions of saposin C have implications for understanding the molecular pathogenesis of Gaucher disease and development of more efficient therapies.

**Study of folic acid pathway genes alteration in fragile X syndrome.** *M. Mirakhory, A. Aleyassin.* National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran.

Fragile X syndrome is one of the most common causes of inherited mental retardation in males after Down syndrome. Penetrance of fragile X syndrome is approximately 70% in male and 30% in female. It is due to the expansion of CGG repeat region and methylation of FMR1 gene. To date less attention was to study important genetic factors that may play role in neuropathology and severity of physical characteristic of fragile X syndrome. Folic acid, has been prescribed in some fragile X cases for reduction of hyperactivity and attention deficit in prepuberty. Folic acid derivatives have important roles in DNA methylation and neural development and function as well as in synthesis of neurotransmitters and influence diverse process in the CNS. Our hypothesis was to study impaired folate metabolism in fragile X patients as the first step to evaluate the linkage between folate metabolism dysfunction and methylation of FMR1 gene. This is the first study to assess this hypothesis by comparing the frequency of three common mutations in C677T, A1298C methyltetra hydrofolate reductase (MTHFR) and A66G methionine synthase reductase (MTRR) genes, key enzymes in folate metabolism, in 34 male fragile X patients and 60 males control. These common mutations are reported to decrease folic acid level and increase plasma homocysteine levels less in carriers and more in homozygote forms. All cases have been initially diagnosed with fragile X syndrome by molecular methods. The statistical analysis showed a significant linkage between C677T MTHFR mutations with fragile X syndrome supported with a P value of 0.021 and chi-square of 7.719. However, no significant correlations were obtained for A1298C MTHFR and A66G MTRR mutations. The P value for A66G was stronger 0.148 compared to A1298C 0.241 that is in harmony with the less effect of A1298C mutation in folate metabolism. Significantly high frequency of C677T MTHFR mutation may suggest effect of folate derivatives on neuropathology and methylation process and more investigations to perform on the role of folate metabolism in fragile X syndrome.

**The first case of parental gonadal mosaicism for Fabry disease: low proportion of mutant DNA in paternal sperm leads to multiple affected offspring.** *R. Dobrovolny<sup>1</sup>, M. Hrebicek<sup>1</sup>, L. Dvorakova<sup>1</sup>, J. Bultas<sup>2</sup>, S. Magage<sup>2</sup>, J.C. Lubanda<sup>2</sup>, J. Ledvinova<sup>1</sup>, H. Poupetova<sup>1</sup>.* 1) Institute of IEM, Praha 2, Czech Republic; 2) Second Department of Internal Medicine, General Faculty Hospital, Praha 2, Czech Republic.

Fabry disease is a common X-linked lysosomal storage disorder caused by alpha-galactosidase A deficiency. We present two sisters with Fabry disease of severity comparable to that of affected males of the same age. Each of the sisters developed abnormal renal functions in the third decade of life, one of them underwent successful renal transplantation due to renal failure. Alpha-galactosidase A activities in white blood cells were decreased (8.1 and 33.7 nmol/mg of protein /hour, control range 39-77 nmol/mg of protein /hour ), globotriaosylceramide excretion in the first of the siblings was increased (6600 nmol/24h). X-inactivation was studied using HUMARA method. Both patients had skewed X-inactivation patterns (90:10 and 80:20, respectively) in favor of the chromosome carrying the mutation. This may explain the severity of the phenotype. Both patients were heterozygous for Q330X mutation in the alpha-galactosidase A gene, while their parents were tested negative for the Q330X in leucocytes, cells from urinary sediment and saliva. We hypothesized that gonadal mosaicism in one of the parents may be the cause of multiple affected offspring. The haplotype analysis showed that the mutant chromosome was of paternal origin. Using a PCR based technique we examined DNA isolated from sperm of the father and found a small fraction of mutant DNA. The mutant DNA formed approximately 5% of total as judged by the proportion of mutant and wild-type PCR products. To our knowledge this is the first case of parental mosaicism in Fabry disease. It underlines the importance to consider gonadal mosaicism when counselling families with diseases with low incidence of gonadal mosaicism.

Program Nr: 1683 from 2003 ASHG Annual Meeting

**Comparison of amino acid concentrations using both HPLC and tandem mass spectrometry.** *R. Eusebio, B. Lim, M. Parkhurst, M. Najjar, T. Giudici, M. Jamehdor, Y. Qu.* Dept. of Genetic Testing, Kaiser Permanente, Los Angeles, CA.

Traditionally, amino acids were quantitated using an amino acid analyzer or high performance liquid chromatography (HPLC). Recently, tandem mass spectrometry (MS/MS) has been used for amino acid analysis to screen for some aminoacidopathies in newborns. To validate amino acid levels measured with MS/MS, a comparison study was initiated using both HPLC and MS/MS from the same plasma sample set.

Ten amino acid concentrations were compared using 50 plasma samples. These amino acids were: alanine, arginine, citrulline, glycine, leucine with isoleucine, methionine, ornithine, phenylalanine, tyrosine and valine. The percent errors using amino acid concentrations from HPLC as theoretical values and those from MS/MS as experimental values were calculated. The lowest percent error was found in citrulline level (1%) and the highest percent error, in arginine concentration (29%). The study demonstrated that levels of these ten amino acids quantitated with MS/MS were comparable to those with HPLC. MS/MS can be used to quantitate at least ten amino acids reliably from plasma samples along with acylcarnitine analysis.

**Congenital disorder of glycosylation type Ib: the second North American patient.** *G.M. Enns<sup>1</sup>, R. Gallagher<sup>1</sup>, S. Conrad<sup>2</sup>, J.F. O'Brien<sup>3</sup>, H.H. Freeze<sup>4</sup>.* 1) Div Med Genet, Stanford Univ, CA; 2) Div Endocrinology, CH Oakland, CA; 3) Mayo Clinic, Rochester, MN; 4) The Burnham Inst, La Jolla, CA.

Congenital disorder of glycosylation type Ib (CDG Ib) is a recently described disorder of glycoprotein metabolism caused by phosphomannose isomerase (PMI) deficiency. Clinical features include hyperinsulinemic hypoglycemia, liver disease, protein-losing enteropathy, and coagulation defects. In contrast to other types of CDG, there is no neurologic involvement. In order to increase awareness of this disorder, we report the clinical and biochemical features of the second patient detected in North America. The pregnancy and neonatal period were normal. She had multiple infections in infancy, including enteritis, a UTI, amoebiasis, and pneumonia. She had a seizure associated with an abnormal EEG at 5 months and she is maintained on anticonvulsant therapy. Hepatomegaly was noted at 9 months. At 3 years, she was hospitalized for seizures and was found to have hepatomegaly, elevated transaminases, and hypoglycemia with hyperinsulinism. Liver biopsy showed periportal fibrosis and dilated and tortuous bile duct lobules. Coagulation studies were normal. Frequent feeds and cornstarch supplementation were started. Mass spectrometry showed a type 1 pattern of abnormal glycosylation (mono- to di-oligo ratio=0.589, n10.074; a- to di-oligo ratio=0.065, n10.022). Leukocyte PMI activity was 0.29 nmol/min/mg (3.1% of a simultaneous normal control). The parents had ~50% normal activity, consistent with carrier status. Mannose therapy was started (150 mg/kg q6h) and the glycosylation profile normalized within four months. No side effects have been observed. The hypoglycemia has resolved and liver disease has not progressed. Seizures are well-controlled and her development is normal. We conclude that CDG Ib is a treatable disorder that is likely underdiagnosed. CDG Ib should be considered in any child with unexplained hypoglycemia and/or liver disease (or other chronic GI illness). Because other CDG Ib patients have had seizures unrelated to hypoglycemia, it is possible that a degree of neurologic involvement is present in CDG Ib. Identification of other cases will be needed to clarify this.

**Mutations in Carbamyl Phosphate Synthetase I (CPSI) inactivate the enzyme in in vitro expressed protein.** A. Eeds<sup>1</sup>, M. Yadav<sup>2</sup>, L. Hall<sup>2</sup>, G. Cunningham<sup>3</sup>, B. Christman<sup>3</sup>, J. Haines<sup>1</sup>, M. Summar<sup>1,2</sup>. 1) Dept Molec Physiology, Vanderbilt Univ, Nashville, TN; 2) Dept Pediatrics/Div Medical Genetics, Vanderbilt Univ, Nashville, TN; 3) Dept Pulmonary Medicine, Vanderbilt Univ, Nashville, TN.

CPS-1 deficiency (CPS1D) is a rare autosomal recessive disorder that causes either a lethal or severe defect in the function of the urea cycle. As part of our mutation studies of the enzyme CPSI, we have used site directed mutagenesis to create mutations in a construct containing the cDNA sequence for CPSI. These mutations, 434Ins6 and G2855T, correspond to those seen in patients. The 434Ins mutation codes for two additional amino acids, glycine and arginine, and is located in the region of CPSI known as the interaction subdomain. The G2855T mutation changes a glycine to a valine and is located in a region of unknown function that is thought to be important for structural conservation. Because even low activities of CPSI seem to be protective, we hypothesized that any mutation causing severe neonatal hyperammonemia in a patient would result in severe CPSI activity deficiency. We used site directed mutagenesis to place both mutations in our CPSI producing vector based on a pcDNA3.1 plasmid. We transiently transfected the CPSI vectors into Cos7 cells, which produce no endogenous CPSI. We tested cell lysates for CPSI enzyme activity 48 hours after transfection. Activities were compared to our normal CPSI expression vector in Cos7 cells and hepatic tissue controls. Though western blots showed the presence of CPSI from all clones, there was no detectable CPSI activity following a colorimetric assay to measure citrulline formation for the mutants. These findings demonstrate the utility of this system for determining the effects of deleterious mutations, and the severity of the changes from a simple missense mutation in this large gene.

**Glycerol kinase (GK) splice site mutations associated with nonsense-mediated decay of mutant RNAs and asymptomatic isolated GK deficiency.** *K.M. Dipple<sup>1,2,3</sup>, Y.-H. Zhang<sup>1</sup>, B.-L. Huang<sup>1</sup>, E.R.B. McCabe<sup>1,2,3</sup>*. 1) Dept Peds, David Geffen Sch Med at UCLA, LA, CA; 2) Dept of Hum Gen, David Geffen Sch Med at UCLA, LA, CA; 3) Mattel Children's Hospital at UCLA, LA, CA.

Isolated glycerol kinase deficiency (GKD) is an X-linked inborn error of metabolism that is either symptomatic or asymptomatic. Symptomatic patients have episodic metabolic crises. We showed previously that GKD is due to deletions of, or mutations within, the GK gene coding for 524 amino acids (aa) and that there is no genotype-phenotype correlation. We identified patients with GKD and sequenced the 20 exons and intron-exon boundaries in their DNA. We identified three patients with GK splice-site mutations and studied their RNA stability. The first had 6.8% of normal GK activity and a mutation, IVS3+1 GA that resulted in deletion of exon 3 (107bp), and a frameshift, predicted to give an 79 aa protein. The second had 8.3% of normal GK activity due to a mutation, IVS4-1 GA, which resulted in deletion of exon 5 (77bp) and a frameshift predicting a 119 aa protein. The third had 5.3% of normal GK activity due to a mutation, IVS10+1 GT, resulting in deletion of exon 10 (43bp) and a frameshift predicting a 307 aa protein. To study the effect of splice-site mutations on RNA species, we performed RT-PCR and found only normal sized products for all three, not the anticipated abnormally spliced forms. We hypothesized that abnormal RNA species were lost by nonsense-mediated decay (NMD). When patients' lymphoblastoid cells were incubated with anisomycin to block NMD for two hours, two RNA species were seen in each patient. Sequence analysis revealed that for each patient the larger bands represented wildtype GK RNAs and smaller bands represented mutant misspliced RNAs. Interestingly, all these individuals presented with the asymptomatic form of GKD. These data suggest that these splice site mutations lead to unstable mRNA species that are not stable enough to be translated. Normal RNA species observed in each patient are likely responsible for their mild phenotypes. We speculate that influences on RNA processing and stability represent modifiers of the GKD phenotype.

**Molecular analysis of carnitine-acylcarnitine translocase deficiency.** *J.H. Ding<sup>1</sup>, J.M. Mallory<sup>1</sup>, B.Z. Yang<sup>1</sup>, A.J. Davis<sup>2</sup>, D. Macgregor<sup>2</sup>, C.R. Roe<sup>1</sup>.* 1) Institute of Metabolic Disease, Baylor University Medical Center, Dallas, TX; 2) Health Care Corporation of St. John's, St. John's, Canada.

The carnitine-acylcarnitine translocase (CACT) deficiency, an autosomal recessive disorder, is one of the most severe defects of mitochondrial fatty acid oxidation, which presents as two clinical phenotypes: a severe neonatal onset form with cardiomyopathy and a milder phenotype with hypoketotic hypoglycemia. In this study, the patient is currently a twenty-year-old college girl of non-consanguineous English/Irish parents. She presented at three months of age with hepatomegaly and hypoketotic hypoglycemia. She had carried a diagnosis of "carnitine deficiency" and was placed on IV carnitine. Recently, incubation of her fibroblasts with [16-<sup>2</sup>H<sub>3</sub>]palmitic acid and analysis by tandem mass spectrometry revealed an increased concentration of [16-<sup>2</sup>H<sub>3</sub>]palmitoylcarnitine, suggesting the diagnoses of either CACT or Carnitine palmitoyltransferase II (CPT II) deficiency. CACT activity in fibroblast was almost absent and confirmed the diagnosis of CACT deficiency. To investigate the molecular basis of CACT deficiency, all of CACT exons, including intron and exon boundaries, were amplified and sequenced. RT-PCR was also performed to amplify CACT coding region. A novel mutation was identified, which was a 842C>T transition in exon 8, resulting in an alanine-to-valine substitution at residue 281 of the protein (A281V). This novel mutation was also verified by a PCR/restriction test. Both parents were also tested and found to be heterozygous for 842C>T. In addition, in our total of 6 unrelated patients with CACT deficiency, the correlation of mutant genotype to clinical phenotype was investigated.

**3-methylcrotonyl-CoA carboxylase deficiency (MCC): molecular analysis including patients detected by tandem mass spectrometry (TMS) based newborn screening.** *M.F. Dantas<sup>1,2</sup>, T. Suormala<sup>1</sup>, B. Fowler<sup>1</sup>, M.R.*

*Baumgartner<sup>1,2</sup>*. 1) Metabolic Unit, University Children's Hospital, Basel, Switzerland; 2) Division of Metabolism and Molecular Pediatrics, University Children's Hospital, Zurich, Switzerland.

Isolated MCC deficiency is an autosomal recessive disorder of leucine catabolism. It appears to be the most frequent organic aciduria detected in TMS based newborn screening programs. Patients with MCC deficiency show elevated urinary excretion of 3-hydroxyisovalerate and 3-methylcrotonylglycine, usually combined with secondary carnitine deficiency. The phenotype is variable, ranging from neonatal onset with severe neurological involvement to asymptomatic adults. MCC is a heteromeric mitochondrial enzyme composed of 2 non-identical subunits, an  $\alpha$ -subunit which binds biotin and a smaller  $\beta$ -subunit. The genes encoding the two subunits, *MCCA* and *MCCB*, have been recently cloned. To date 8 *MCCA* and 12 *MCCB* mutant alleles have been reported. We report detailed molecular analysis in a further 17 MCC deficient probands. All showed characteristic abnormal organic acid excretion and severely deficient MCC activity (0-18% of control) was confirmed in cultured fibroblasts. 6 probands were diagnosed because of clinical symptoms, and 11 were asymptomatic newborns detected by TMS based newborn screening. To detect mutant alleles we amplified patient cDNA from fibroblast RNA, sequenced the product directly and confirmed all identified mutations by amplifying and sequencing of genomic DNA. Six of the probands revealed mutations in *MCCA*, and 11 in *MCCB*. We identified 2 new *MCCA* and 8 new *MCCB* mutant alleles including 3 missense, 3 nonsense, 3 splice mutations and 1 mutation which causes the addition of 3 amino acids to the C-terminus of the  $\beta$ -subunit. To test the functional consequences of the missense mutations we transfected all missense alleles into reference MCC-deficient transformed cell lines and measured MCC activity. All missense mutations but one result in loss of function.

Our data demonstrate no clear correlation between genotype and phenotype, suggesting that factors other than the MCC loci must have a major influence on the phenotype of MCC deficiency.

**LDL RECEPTOR GENE MUTATIONS AND LIPID PATTERN IN ITALIAN CHILDREN AFFECTED BY FAMILIAL HYPERCHOLESTEROLEMIA.** *O. Guardamagna, C. Bondone, C. Allora, V. Molini, I. Rabbone.*  
Pediatrics, Univ de Torino, Torino, Italy.

**Background:** Familial Hypercholesterolemia (FH) is a genetic disorder of lipid metabolism caused by different mutations in LDL-receptor (LDLR) gene. **Aim of this study** is to correlate different mutations of LDLR gene in FH children to lipoprotein pattern. **Methods:** we have recruited 18 affected children among patients afferring to our clinic (age 93 ys). Each subjects was analyzed by screening the LDLR gene and lipid profile was tested in all children: total cholesterol (TC), HDL-cholesterol, tryglicerides were evaluated with enzymatic method; apolipoprotein B (apoB) with immunoturbidimetric method. **Results:** we identified 10

LDLR/mutation	exon	TCmg%	LDL-Cmg%	APOBmg%	N
Y468C (new)	10	348	286	170	1
Fs354Term360	8	351	279	170	1
1075delAGGATC (new)	8	352	278	159	1
G528D	11	342	267	171	4
E397STOP (new)	9	335	236	148	2
D558Y	12	275	204	139	3
D200G	4	334	203	136	1
V510I (new)	10	296	191	131	1
W469R (new)	10	234	186	125	1
IVS11-1076 10G>A	in11	229	153	115	1
IVSnt-8T>C	in7	225	126	107	1
Q12STOP	2	228	135	91	1.

**Conclusion:** we observed large variation of lipid levels among children with different mutation of LDLR gene, 5 of which were firstly detected. Further study will be necessary to define the relationship between LDLR mutations, biochemical profile and cardiovascular risk.

**Incidence of Hyperphenylalaninemia in Louisiana: 1985-2000.** *H. Andersson<sup>1</sup>, L. Burrage<sup>1</sup>, C. Myers<sup>2</sup>, A. Hagar<sup>3</sup>, H. Bradford<sup>3</sup>, J. Miller<sup>1</sup>.* 1) Hayward Gen Ctr, Tulane Univ Medical Ctr, New Orleans, LA; 2) Louisiana Genetic Disease Program; 3) Louisiana Newborn Screening Lab.

**Purpose:** No previous study has determined the incidence of variant phenylketonuria (untreated plasma phenylalanine 2-20mg/dl). Additionally, the incidence of classical phenylketonuria (PKU) is based primarily on studies from northern European populations, a predominantly white population. Bickel (1981) estimated the incidence of PKU in Europe as 1:9400. No longitudinal study has established the incidence of PKU in the American population. This study examines the epidemiology of hyperphenylalaninemia in Louisiana (LA) in 1,106,909 births over a 15 year period (1985-1999). **Methods:** Complete ascertainment of LA-born patients with hyperphenylalaninemia was obtained thru the Newborn Screening Program of the LA Genetic Diseases Program and the Hayward Genetics Center, the metabolic disease referral center for LA. All positive PKU newborn screens (Guthrie) were referred for confirmatory plasma amino acids. Plasma PHE levels above 2mg/dl were considered abnormal and all such patients were evaluated and followed for hyperphenylalaninemia. **Results:** The overall incidence of hyperphenylalaninemia in LA was 1:15,849. The incidences of classical and variant phenylketonuria (PKU) were 1:18,365 and 1:22,446, respectively, in the white population and 1:113,966 and 1:227,933, respectively, in the non-white population. One patient with 6-pyruvoyl BH<sub>4</sub> synthase deficiency was identified (1:1,061,909). **Conclusion:** The incidences of classical and variant PKU were much higher in the white than in the non-white population but were generally much lower than in the European population. The incidence of classical PKU in the Louisiana African-American population appears to be lower than estimated in Maryland (1:50,000; Hofman et al, 1991). An unusually high incidence of PKU was found in the Acadian area of LA. These figures allow more accurate risk analysis in whites and non-whites for hyperphenylalaninemia.

**Functional consequences of *PRODH* missense mutations associated with hyperprolinemia and/or schizophrenia (SZ).** H.U. Bender<sup>1,2</sup>, S. Almashanu<sup>1,2</sup>, G. Steel<sup>1,2</sup>, A. Pulver<sup>3</sup>, D. Valle<sup>1,2</sup>. 1) Inst Genetic Medicine; 2) Howard Hughes Medical Institute; 3) Dept of Psychiatry, Johns Hopkins Univ, Baltimore, MD.

*PRODH* maps to 22q11 at the centromeric end of the region frequently deleted in DiGeorge Syndrome. *PRODH* encodes proline oxidase (POX) a mitochondrial inner membrane enzyme that catalyzes the first step in the proline degradation pathway and is expressed in liver, kidney and brain. At least 16 *PRODH* missense mutations have been reported, some proposed to be associated either directly or through linkage disequilibrium with increased risk for SZ (Jacquet et al 2002;Liu et al 2002). *PRODH* mutations have also been described in 3 patients with type I hyperprolinemia (Humbertclaude et al 2001;Jacquet et al 2002). The functional consequences of these mutations have been inferred by evolutionary conservation but none have been tested directly. We report the functional consequences of 16 *PRODH* missense mutations. We mutagenized *PRODH* cDNA constructs, transfected them into CHO-C9 cells and assayed POX specific activity. We found 4 alleles (R185Q, L289M, A455S, A472T) with a mild (<30%); 6 (Q19P, A167V, R185W, D426N, V427M, R431H) with a moderate (30-70%) and 5 (P406L, L441P, R453C, T466M, Q521E) with severe (>70%) reduction in POX activity. Interestingly one (Q521R) had the opposite effect, increasing POX activity to 120% of control. In a recent publication, 6 of these mutations (L289M, R431H, L441P, R453C, A455S, Q521R) have been reported in hyperprolinemic patients, some with SZ (Jacquet et al 2002). The activity of these alleles ranges from 0-120% of controls. Three alleles (V427M, R453C, A472T) showed significant association with SZ in populations of distinct ethnic origin with no assessment of proline levels (Liu et al 2002). In our results, POX activity encoded by these alleles ranges from a mild to severe reduction. In summary we find 1) *PRODH* alleles associated with severe hyperprolinemia have <10% enzyme activity; 2) alleles with mild to moderate reduction show no direct association with plasma proline levels; and 3) alleles associated with SZ vary widely in their POX activity, suggesting alterations in POX activity per se do not affect susceptibility.

**Screening for metabolic disorders using tandem mass spectrometry.** *F.W. Lorey, G.C. Cunningham, J. Sherwin, L. Feuchtbaum, B. Currier, K. Velazquez, S. Levine.* Genetic Disease Branch, CA Dept Health Services, Richmond, CA.

California initiated a large-scale research project to investigate the feasibility of testing for multiple metabolic disorders by tandem mass spectrometry. Five tandem mass spectrometers were installed in the state laboratory and a dedicated database was developed. An informed consent was developed and the research plan was approved by the state IRB. From January 7, 2002 through June 13, 2003, there were 331,217 infants screened. Cutoffs for 42 analytes were determined through statistical analysis, workload considerations, and disease-specific factors. They were revised twice after gathering additional data and with input from metabolic specialists. The initial positive rate was 0.49% and final rate was 0.08%. One diagnosed case was confirmed for every 10 positive screens referred. Specificity varies by disorder and analyte. The results are as follows:

MCADD	SCADD	MMA/PA	GA-II	3-MCC	Arg	MSUD	Total
11	18	10	1	2	1	1	44
1/30,000	1/18,400	1/32,000	1/331,000	1/165,600	1/331,000	1/165,600	1/7500

Three cases were missed, including one MSUD included in the prevalence rate, one VLCADD not detectable because of treatment, and one LCHADD. Both the MSUD and the LCHADD would be detected by our latest cutoffs. Cases not screened were reported to us and they were tested subsequently. Older cases prior to the study were tested by pulling frozen stored newborn specimens. Clinical data are being collected on diagnosed cases. This methodology successfully detects most cases of metabolic disease and can prevent serious morbidity by initiating treatment before serious symptoms appear.

**Reverse-hybridization assay for mutations associated with hereditary sugar intolerance.** *G. Kriegshaeuser<sup>1</sup>, W. Krugluger<sup>2</sup>, F. Kury<sup>1</sup>, C. Oberkanins<sup>1</sup>*. 1) ViennaLab Labordiagnostika GmbH, A-1110 Vienna, Austria; 2) Department of Clinical Chemistry, Municipal Hospital Rudolfstiftung, Vienna, Austria.

Dietary carbohydrates for humans include polysaccharides (starch), disaccharides (sucrose, lactose) and monosaccharides (glucose, fructose, galactose). During digestion, specific enzymes will initially hydrolyze poly- and disaccharides into their monosaccharide constituents, which then become absorbed by apical cells of the small intestine and further metabolized. A variety of genetically determined enzyme and transporter deficiencies may cause hereditary intolerance to common dietary sugars. Lactose intolerance (adult-type hypolactasia, lactase non-persistence) is an extremely frequent autosomal recessive condition causing diarrhea, nausea and flatulence. It is highly associated with two mutations located upstream from the lactase-phlorizin hydrolase (LPH) gene locus. Hereditary fructose intolerance is an autosomal recessive disorder caused by mutations in the aldolase B gene. Affected subjects suffer from severe abdominal pain, vomiting, hypoglycaemia, and unless fructose-containing food is strictly avoided may even die from irreversible damage of the liver and kidney. We have developed a reverse-hybridization assay for the rapid and simultaneous detection of two mutations (-13910 C/T, -22018 G/A) upstream to the LPH gene and three mutations (A149P, A174D, N334K) in the aldolase B gene. The test is based on multiplex DNA amplification and hybridization to a teststrip presenting a parallel array of allele-specific oligonucleotide probes for each mutation. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours, and hybridization/detection may be carried out manually or essentially automated using existing instrumentation (e.g. TECAN profiBlot). The test is simple and convenient, requires very small amounts of samples, and can easily be modified to include additional mutations. (oberkanins@viennalab.co.at).

**Somatic mosaicism in a male with an exon skipping mutation in the E1 subunit (*PDHA1*) of the pyruvate dehydrogenase complex results in a mild phenotype.** *K. Okajima*<sup>1,2</sup>, *G.C. Nickel*<sup>2</sup>, *E. Sieverding*<sup>1</sup>, *M.L. Warman*<sup>2</sup>, *D.S. Kerr*<sup>1</sup>. 1) CIDEM, Rainbow Babies & Children's Hospital; 2) Genetics, Case Western Reserve University, Cleveland Ohio.

The pyruvate dehydrogenase complex is essential for aerobic glucose metabolism. Hemizygous, complete loss-of-function mutations of the E1 subunit, (*PDHA1*) which is encoded on chromosome Xp22, are presumed to be lethal in males. In females with heterozygous loss-of-function mutations, a range of phenotypes have been reported, attributable to X-inactivation. We evaluated a 5 year old male with hypotonia, moderate developmental delay and seizures, but normal growth and brain MRI. His lactate was normal to mildly elevated in blood (1.5-2.9mM) and CSF (3.5-4.4mM) with a normal lactate/pyruvate ratio. PDC activity was 27% of controls in skin fibroblasts, 37% in skeletal muscle and 60% in lymphocytes. *PDHA1* cDNA analysis from skin fibroblasts revealed two populations of mRNA; one species was wild type, the other lacked exon 6. Genomic DNA from fibroblasts contained nearly equal amounts of wild-type sequence and sequence harboring g.592GA in exon 6. Flanking introns had no mutation. This 592G is conserved among species, including yeast. This change is predicted to create a novel SRp40 exonic splice enhancer site. Klinefelter syndrome, chimerism or trisomic rescue were excluded as possible explanations for the existence of two X-linked alleles by a normal karyotype (46XY) and a single DNA complement on polymorphic marker analysis. Immunohistochemistry of skin fibroblasts appears mosaic for *PDHA1*. These data indicate that this child is a somatic mosaic for a non-functional *PDHA1*. A lower percentage of mutant genomic DNA in EBV-transformed lymphoblasts implies tissue variation. This is the second report of a *PDHA1* exon 6 skipping mutation in viable males, the first being in two male cousins with Leigh syndrome and a silent mutation that caused partial skipping of exon 6. Somatic mosaicism for a non functional *PDHA1* has also been reported in a newborn male with lethal lactic acidosis. In our patient, the existence of normal mRNA due to somatic mosaicism accounts for his mild phenotype.

**One year experience in diagnosis of inborn errors of metabolism using tandem mass spectrometry.** *B. Lim<sup>1</sup>, R. Eusebio<sup>1</sup>, J. Kane<sup>1</sup>, T. Giudici<sup>1</sup>, M. Najjar<sup>1</sup>, R. Mardach<sup>2</sup>, M. Lipson<sup>3</sup>, J. Baker<sup>3</sup>, K. Johnston<sup>3</sup>, M. Jamehdor<sup>1</sup>, Y. Qu<sup>1</sup>.*  
1) Dept. of Genetic Testing, Kaiser Permanente, Los Angeles, CA; 2) Regional Metabolic Service, Kaiser Permanente, Los Angeles, CA; 3) Dept. of Genetics, Kaiser Permanente Medical Center, Oakland, CA.

Tandem mass spectrometry (MS/MS) has been used for diagnostic work-up for fatty acid oxidation disorders (FAODs), some aminoacidopathies and organicacidopathies. Here we report four inborn errors of metabolism (IEM) diagnosed using MS/MS in one year of time starting March 2002. These diagnoses were made based on the accumulation of diagnostic acylcarnitines and abnormal compounds detected in urine organic acid analyses (UOAA). These IEMs are: medium chain acyl-CoA dehydrogenase deficiency (MCAD), methylmalonic aciduria (MMA), cobalamin C defect (cbl C), and glutaric aciduria type I (GA I).

In MCAD patient, acylcarnitine profile showed increased C8-acylcarnitine (10 times normal value) with more than 700 times elevated suberylglycine in UOAA. In MMA patient, C3- acylcarnitine was elevated 12 times of normal level with highly elevated methylmalonic, methylcitric and 3-hydroxypropionic acids in UOAA. In cbl C patient, C3-acylcarnitine was increased 8 times of normal level with elevated methylmalonic acid, total homocysteine and decreased methionine. In GA I patient, C5-DC- acylcarnitine was twice as high as normal control with highly elevated glutaric and 3-hydroxyglutaric acids. Two hundred fifty samples were received for acylcarnitine analysis from March 2002 to March 2003. The diagnostic rate is 1.6% (4 out of 250). Acylcarnitine analysis and UOAA are a good pair of complementary tests for diagnosis of FAODs and organicacidopathies.

**Mutation and biochemical analysis of patients belonging to the *cblA* complementation class of vitamin B<sub>12</sub>-**

**dependent methylmalonic aciduria.** *J.P. Lerner-Ellis*<sup>1</sup>, *C.M. Dobson*<sup>2</sup>, *T. Wai*<sup>1</sup>, *D. Watkins*<sup>1</sup>, *J.C. Tirone*<sup>1</sup>, *C. Dor*<sup>3</sup>, *P. Lepage*<sup>3</sup>, *R.A. Gravel*<sup>2</sup>, *D.S. Rosenblatt*<sup>1,4,5</sup>. 1) Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Biochemistry and Molecular Biology, University of Calgary, Alberta, Canada; 3) McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada; 4) Biology and Medicine, McGill University, Montreal, Quebec, Canada; 5) McGill University Health Centre, Montreal, Quebec, Canada.

Methylmalonic acidemias (MMA) are rare human disorders caused by deficient activity of the mitochondrial methylmalonyl CoA mutase enzyme (MCM). MCM catalyzes the vitamin B<sub>12</sub> (cobalamin, Cbl)-dependent rearrangement of L-methylmalonyl CoA to succinyl CoA and uses adenosylcobalamin (AdoCbl) as cofactor. The *cblA* complementation class of inborn errors of cobalamin metabolism is one of three known MMA disorders uniquely involved in AdoCbl synthesis. The gene responsible for *cblA* has been identified through the examination of prokaryotic gene arrangements and is called *MMAA*. The precise role of the *MMAA* gene product is not known, but previous studies have suggested its involvement in mitochondrial Cbl transport. By sequencing and restriction endonuclease enzyme analysis thirteen novel mutations in the *MMAA* gene of *cblA* patients were identified. Four previously identified mutations in *MMAA* were confirmed. In total seventeen mutations have been identified: eleven result in premature stop codons including two deletions and two insertions, two are splice site defects, and four are missense mutations that occur at highly conserved residues. Forty patients were analyzed. In twelve patients, two causal mutations were identified; in eleven patients, a single mutation was identified. In the remaining seventeen patients, no *MMAA* coding region sequence changes were identified. None of the sequence changes identified in *cblA* patients were found in one hundred alleles from unrelated control individuals. The biochemical profiles of patient fibroblast cell lines were examined for their ability to incorporate radio labeled [<sup>14</sup>C]-propionate into macromolecules and for the distribution of Cbl derivatives in cell extracts as compared to normal fibroblasts.

**Phenotypic characterization of glycerol kinase loci in *Drosophila melanogaster*.** J.A. Martinez<sup>1,2</sup>, E.R.B. McCabe<sup>1,2</sup>.  
1) Department of Pediatrics, David Geffen School of Medicine at UCLA and Mattel Children's Hospital at UCLA, Los Angeles, CA; 2) Department of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA.

Glycerol kinase (GK) deficiency (GKD) is an X-linked, phenotypically heterogeneous metabolic disorder with variable severity of presentation among individuals with similar genotypic mutations. We propose to attempt to understand this variability in phenotypic expression and to dissect its molecular basis by investigating GK in the model organism *Drosophila melanogaster*. We analyzed the fully sequenced fruit fly genome and this revealed two loci with significant sequence homology to mammalian GK at the protein level. Alignment of these sequences revealed conservation of key residues required for GK activity, as well as motifs for protein tyrosine and serine-threonine phosphorylation. In addition, multiple DNA-binding elements for liver- and muscle-specific transcription factors were found to be conserved within 2 kb upstream of their transcriptional start sites. These findings strongly suggest an evolutionarily conserved mode of regulation, both at the transcriptional and post-translation levels. Glycerol kinase transcripts were expressed at high levels during larval stages, particularly in the fat body, the *Drosophila* equivalent of the mammalian liver. P-element mediated mutagenesis of GK loci leads to recessive lethality, demonstrating an essential role of GK in development of this model organism. We are presently characterizing the role of several conserved signaling pathways as potential modifiers of glycerol kinase phenotypes. We speculate that investigation of GK in *Drosophila* will inform us about the role of this enzyme in development and will provide us with a tool to examine genetic modifiers of the GKD phenotype.

**Expression of alternatively spliced glycerol kinase is altered during development.** *R. Ohira*<sup>1</sup>, *E.R.B. McCabe*<sup>1,2</sup>. 1) Dept Hum Gen, David Geffen Sch Med at UCLA, LA, CA; 2) Dept Peds, David Geffen Sch Med and Mattel Children's Hospital at UCLA, LA, CA.

Glycerol kinase (GK) is an enzyme involved in fat and carbohydrate metabolism. Mutations in *GK* result in GK deficiency. Targeted deletion of murine glycerol kinase (*Gyk*) results in *Gyk*-deficient male mice that are normal at birth, then show postnatal growth retardation, hyperglycerolemia, elevated free fatty acids, and death by 3-4 days after birth. Two alternatively spliced forms of GK have been identified: GK with exon 18 (GK+EX18) in human fetal brain and testes, and GK without exon 18 (GK-EX18) in liver. We investigated differences in *GK/Gyk* expression during development. Northern blot analysis with a *Gyk* probe was performed on a murine blot containing RNA from different embryonic stages: 7, 11, 15 and 17 days post coitum. Two transcripts were seen at all stages, with an increase in expression in the latter stages of development. Reverse transcriptase-polymerase chain reaction (RT-PCR) amplification using primers to human Xp21 GK sequence within exon 16 and 3-untranslated region resulted in a 280 bp product for GK+EX18 or a 193 bp product for GK-EX18. RT-PCR on human fetal and adult liver poly-A RNA showed that fetal liver expressed both forms with GK-EX18 more intense; however, adult liver only expressed GK+EX18. RT-PCR using primers specific to murine *Gyk* was carried out on total RNA extracted from fat, heart, liver, kidney, and brain of an adult mouse. *Gyk* could not be detected in fat. Liver expressed only *Gyk*-EX18, heart expressed both at equal intensity, kidney expressed both with *Gyk*-EX18 greater, and brain expressed both with *Gyk*+EX18 greater. We described similar expression patterns in humans. Two splice forms were transfected into Cos7 cells and assayed for GK activity; preliminary results suggest GK+EX18 is compartmentalized. *Gyk* expression increases during embryonic development, and at least in liver, proportions of the alternatively spliced forms of GK vary during development. Preliminary data suggest GK+EX18 may be recruited to a cellular compartment. We speculate that these alternatively spliced forms of GK may have different functions within the cell.

**Essential Fatty Acid Deficiencies in Metabolic Disorders.** *K. Moseley*<sup>1</sup>, *S. Yano*<sup>1</sup>, *A. Moser*<sup>2</sup>. 1) Medical Genetics, Pediatrics, Childrens Hospital Los Angeles, Los Angeles, CA; 2) Kennedy Krieger Institute, Baltimore, MD.

**INTRODUCTION:** Abnormally low blood levels of docosahexaenoic acid (DHA) and arachidonic acid (AA) have been reported in children with phenylketonuria (PKU) who have been following a phenylalanine-restricted diet with supplementation of a medical product. The reduced levels have been attributed to the fact that the phenylalanine-restricted diet provides only small amounts of animal fats which are the main dietary source of long chain polyunsaturated fatty acids (LCPUFA). We conducted fatty acid analyses in 27 adults/adolescents with PKU, demonstrating reductions in LCPUFA including DHA and AA. We expanded the study to include the pediatric age group of PKU and other metabolic disorders.**METHODS:** 28 pediatric subjects with various metabolic disorders including PKU were surveyed to determine their fatty acid status. Plasma vitamin E and carnitine levels were also evaluated.**RESULTS:**1)In the pediatric age group of the PKU subjects, LCPUFA profiles were similar those obtained in the prior study in adult PKU subjects. The results revealed low AA and DHA acid and elevated docosapentaenoic acid (DPA).2)Subjects with Methylmalonic Acidemia and Propionic Acidemia show slightly elevated DPA ( $p=0.10$ ) although DHA and AA levels were not significantly lower than the controls.3)The two subjects with Malonic Aciduria show a similar profile to PKU subjects with elevated DPA and low AA and DHA.4)The Very Long Chain Acyl-CoA Dehydrogenase deficiency (VLCAD) subjects showed elevated alpha linolenic acid and DPA yet, all the other LCPUFA were not significantly different from the controls. 5)The Vitamin E status was normal in all subjects and the plasma carnitine showed no significant differences in the PKU population.**CONCLUSION** The abnormal fatty acid profiles seen in PKU may account for the subtle cognitive impairments seen in treated PKU individuals. Since DHA and AA have important physiological roles particularly in heart and skeletal muscle as well as in development of brain and retina, supplementation of DHA and AA may prove beneficial in preventing neurological and developmental abnormalities in PKU as well as other metabolic disorders.

**Human Coproporphyrinogen Oxidase (CPO): Biochemical Characterization of Wild-Type Enzyme and Its Naturally Occurring Mutant Forms.** I. Mikula<sup>1</sup>, E. Flachsova<sup>1</sup>, R. Rosipal<sup>1</sup>, B. Demeler<sup>2</sup>, J.-M. Camadro<sup>3</sup>, J. Zeman<sup>1</sup>, J.-C. Deybach<sup>4</sup>, C.S. Raman<sup>2</sup>, P. Martasek<sup>1</sup>. 1) KDDL, 1st Faculty of Medicine, Charles University, Prague, Czech Republic; 2) University of Texas, San Antonio, TX, USA; 3) Institut Jacques Monod, Paris, France; 4) INSERM U409, Paris, France.

Heme acts as a prosthetic group for essential hemoproteins. CPO [E.C.1.3.3.3.] catalyzes the sixth step in heme biosynthesis. The inherited deficiency of CPO causes hereditary coproporphyria (HC), inherited in an autosomally dominant fashion. To better understand heme biosynthesis and address the molecular pathology of HC, we studied recombinant wild-type CPO (using a glutathione-S-transferase expression system) and two naturally occurring mutants (R331W and del390Gly). Wt-CPO and mutated-CPO were purified to electrophoretic homogeneity with specific activities of 4265 nmol/hr/mg protein (wt-CPO), 2100 nmol/hr/mg protein for R331W-CPO and a residual (<1%) activity for del390Gly-CPO. In its active form, human CPO is dimeric. According to the hydrodynamic properties derived from analytical ultracentrifugation, CPO has a nearly globular shape. Both mutation of CPO did not affect dimer formation and sedimentation properties, but led to a significant amount of tightly bound coproporphyrin (R331W), respectively no bound of coproporphyrin (del390Gly). The catalytic efficiency (Kcat/Km) of the R331W-CPO was four-fold lower than wt-CPO, the pI for R331W was 5.56 as compared to 6.40 for wt-CPO, but pH optima were similar (6.8 for wt-CPO, 6.6 for R331W-CPO). Similar characterization is in progress for the whole spectrum of naturally occurring mutants, which, together with our crystallization trials, will add to our knowledge of the molecular pathology of HC and heme biosynthesis. [*Supported by Grant from MSMT of Czech Republic (LN 00A079) and grant Barrande*].

**Novel Mutations in the Fumarylacetoacetase Gene Associated with Tyrosinemia Type I.** *J.-Y. Huang<sup>1,2</sup>, D. Schranz<sup>2</sup>, R. Jack<sup>2</sup>, L. Sniderman-King<sup>1</sup>, S.-H. Chen<sup>1,2</sup>, C.R. Scott<sup>1,2</sup>.* 1) Department of Pediatrics, University of Washington School of Medicine, Seattle, WA; 2) Childrens Hospital and Regional Medical Center, Seattle, WA.

Tyrosinemia type I is a rare autosomal recessive disease of tyrosine metabolism caused by a deficiency in fumarylacetoacetase (FAH), the last enzyme within the tyrosine catabolic pathway. In certain populations the incidence of tryrosinemia I is greater than the estimated incidence of 1/100,000 newborns seen in the United States. This has occurred because of apparent founder effects with the introduction of common mutations in specific populations: IVS12+5g>a in French Canadians and northern Europeans, W262X in Finland, IVS6-1g>t in the Mediterranean population and G337S in Scandinavia. We have screened for gene mutations in fifteen patients with tyrosinemia type I within the U.S. We have used PCR amplification followed by denaturing HPLC and DNA sequencing. In this U.S. population, the four most common mutations worldwide were identified in about half of the alleles and four novel mutations were found that represent previously unrecognized nucleotide alterations. These new mutations are: (1) M202R (c.605T>G), (2) P261L (c.782C>T), (3) IVS7-6t>g, and (4) c.1116-1123 deletion CATAGACC. The first two are missense mutations, the third involves a presumed splicing error, and the 8 bp deletion causes a premature stop codon. Thus, the clinical expression of Tyrosinemia I in the U.S. represents the ethnic diversity of the population without a strong founder effect.

**Four metabolic disorders in a genetic isolate: Population screening and disease prevention.** *M. Khayat<sup>1</sup>, H. Shapira<sup>1</sup>, N. Kfir<sup>1</sup>, I. Gurevitz<sup>1</sup>, H. Mandel<sup>2</sup>, T.C. Falik-Zaccat<sup>1</sup>.* 1) Med Genet, Western Galilee Hospital, Naharia; 2) Dep. of Pediatrics, Rambam Medical Center, Haifa, Israel.

The Druze in Israel represents a unique ethnic community that lives in cultural and geographical isolation in several villages in northern Israel and presents high level of consanguineous marriages. We have identified four rare metabolic disorders in a Druze village including Cerebrotendinous Xanthomatosis (CTX), Prolidase deficiency (PD), arginosuccinic aciduria (ASA) and carbamyl phosphate synthetase deficiency (CPSD). The village consists of approximately 13,000 inhabitants that have originated from a small number of founders. The causative mutation of each disease was identified. Affected individuals were found homozygous for these mutations. These diseases represent a major cause of mortality and morbidity in the village. The aim of this study is to develop an effective carrier screening strategy to reduce the prevalence of genetic disorders in this population. We have screened 190 random healthy individuals for 4 causative mutations and found 12 carriers of CTX (1/16), 9 carriers of PD (1/20), 9 carriers of ASA (1/20) and 3 carriers of CPSD (1/60). Three individuals were carriers for both CTX and PD. Thus, the 30 identified carriers represent a carrier frequency of 1/6 for these rare metabolic disorders. The spouse of each carrier was approached for genetic counseling and testing. Twenty-three agreed to be tested (80%), and none was found to be a carrier for the same disease. Six individuals (20%) refused genetic counseling and testing and 1 was not reached yet. Our results indicate that carrier screening for IEM is warranted and medically indicated in this village. Similar villages exist and should be screened as well for specific frequent diseases in order to reduce the prevalence of genetic disorders among the Israeli Druze. Such screening programs will facilitate targeted genetic counseling, better planning of the special needs for each population, and reduction of the heavy burden from the families and health services. Development of population specific attitudes will improve the compliance of individuals at risk despite cultural and religious restrictions.

**Frequency of the P479L mutation causing Carnitine Palmitoyltransferase (CPT) 1A Deficiency in the Canadian Inuit.** *A.M. Stier<sup>1</sup>, L.E. Seargeant<sup>1</sup>, C. Prasad<sup>1</sup>, D. Grewar<sup>1</sup>, A. Chan<sup>2</sup>, F. Bamforth<sup>2</sup>, P. MacLeod<sup>3</sup>, L. IJlst<sup>4</sup>, R.J.A. Wanders<sup>4</sup>, C.R. Greenberg<sup>1</sup>.* 1) Biochemistry and Med. Genetics, University of Manitoba, Winnipeg, Canada; 2) University of Alberta, Edmonton, Canada; 3) University of Victoria, Victoria, BC, Canada; 4) University of Amsterdam, The Netherlands.

**Objective:** To determine the distribution of CPT1A P479L & CPT2 F352C genotypes for CPT1A deficiency and CPT2 deficiency in 3 Aboriginal kindreds. **Methods and Results:** A 33 week Inuit baby was born to a mother with AFLP (Innes *et al*, 2000). The neonate had transient hypoglycemia and was tested for fatty acid oxidation disorders. Whole blood palmitate oxidation (Seargeant *et al*, 1999) was low. With absent CPT1A enzyme activity in fibroblasts, homozygosity for the P479L mutation, and normal CPT2 activity, the child was diagnosed with CPT1A deficiency. Second and third children have had episodes of hypoketotic hypoglycemia, are homozygous for P479L, have near zero enzyme activity of CPT1A, and are also CPT2 F352C homozygotes, identified because of low CPT2 enzyme activity in the second child. A fourth child, healthy to date, is also homozygous for both mutations. Of 23 extended family members studied, 18 are homozygous and 5 heterozygous for P479L. Five are homozygous, 11 heterozygous, and 7 normal for F352C. Unrelated Inuit identical twins, only one with hypoglycemia, are also homozygous for CPT1A P479L; the F352C CPT2 mutation is absent. Seven asymptomatic 1<sup>st</sup> degree relatives of the twins are homozygous for P479L and none have the F352C mutation. A First Nations man from BC presented with recurrent rhabdomyolysis, reduced CPT1A and normal CPT2 activities and is also homozygous for P479L (Brown *et al*, 2000); he is heterozygous for the F352C mutation. **Conclusions:** The P479L mutation in the CPT1A gene correlates well with near zero fibroblast enzyme activity. An unexpectedly high number of individuals from both Inuit families are homozygous for CPT1A deficiency. This suggests P479L may be a mild mutation or may not result in symptomatic disease as previously thought. Molecular interactions between *CPT1A*, *CPT2*, and other intermediates, as well as epigenetic factors, may thus modulate the severity of CPT1A deficiency.

**Molecular genetic analysis of 20 Korean patients with Glycogen- Storage Disease Type 1.** *S.H Van<sup>1</sup>, C.S. Ki<sup>1</sup>, S.Y. Lee<sup>1</sup>, J.K. Seo<sup>2</sup>, J.W. Kim<sup>1</sup>.* 1) Dept Laboratory Medicine, Samsung Medical Ctr; 2) Dept Pediatrics, Seoul National University, College of Medicine, Seoul, Korea.

Glycogen-storage disease type 1 (GSD-1), also known as "von Gierke disease," is caused by a deficiency in microsomal glucose-6-phosphate activity. Mutations of the glucose-6-phosphatase gene (*G6PC*) are responsible for the most frequent form of GSD-1, the subtype 1a, while mutations of the glucose-6-phosphate transporter gene (*G6PT1*) have been shown to cause the non 1a forms of GSD, namely the 1b and 1c. To elucidate a spectrum of the *G6PC* gene mutations in Korean, we analyzed mutations in 20 unrelated Korean patients suspecting GSD-1 by direct DNA sequencing of the *G6PC* gene. All patients showed the typical clinical symptoms of GSD-1, but only 12 patients were confirmed by enzyme assay for the liver biopsy. Thec.727GT was found to be the major cause of GSD-1, accounting 21 of 40alleles. All 11 patients identified alleles mutations were confirmed by the enzyme assay and of these patients, 10 patients were homozygotes for c.727GT mutation and one is compound heterozygote with different mutations in the two *G6PC* alleles(c.727GT/c.611CG). Thec.611CG, converting a proline to an alanine at codon 178 was novel mutation. One patient showed no mutation in this gene was presented with neutropenia and inflammatory bowel disease in addition to typical clinical symptoms of GSD. We then analyzed mutation of *G6PT1* gene because we suspected clinically GSD-1b. Direct DNA sequencing of the *G6PT1* gene revealed the patient to be a compound heterozygote of a novel missense mutation, c.443CT, and another deletion mutation, c.1042-1043delCT, which has been reported previously. Here, we present that the c.727GT was the most prevalent among Korean GSD-1 patients with the allele frequency of 87.5%(21/24 mutant alleles confirmed by enzyme assay) and two novel missense (c.611CG, c.443CT) mutations were identified in the *G6PC* and *G6PT1* gene, respectively. So we suggest that noninvasive molecular genetic analysis for the c.727GT mutation can be a initial diagnostic tool for patients clinically suspected of having GSD-1 in Korean as an alternative to enzyme assay that requires liver biopsy.

**Fatal Neonatal Presentation of Glycogen Storage Disease Type IV in Two Infants.** *S.K.H. Tay<sup>1</sup>, H.O. Akman<sup>1</sup>, W. Chung<sup>1</sup>, M.G. Pike<sup>2</sup>, A.P. Hays<sup>1</sup>, K. Anyane-Yeboa<sup>1</sup>, S. Shanske<sup>1</sup>, S. DiMauro<sup>1</sup>.* 1) Neurology, Columbia University, NY, NY; 2) Department of Pediatrics, Oxford, UK.

Glycogen storage disease type IV (GSD IV) or Andersen Disease is an autosomal recessive disorder due to deficiency of glycogen branching enzyme (GBE). The GBE1 gene is located on chromosome 3p14 and consists of 16 exons. GSD IV exists in multiple clinically, biochemically, and molecularly heterogeneous forms. The typical form of GSD IV is characterized by rapidly progressive liver cirrhosis and death in childhood. Other variants include a cardiopathic form of childhood, a relatively benign myopathic form of young adults, and a late-onset neurodegenerative disorder (adult polyglucosan body disease, APBD). A severe neuromuscular variant resembling Werdnig-Hoffmann disease had been genetically characterized in only two patients. We describe two more infants, who presented with severe hypotonia and GBE deficiency and harbored novel mutations in the GBE1 gene. Branching enzyme assay, Western blot, RT-PCR and sequencing were performed on both patients. The cDNA of Patient 1 was subcloned and sequenced to delineate the mutations. Muscle biopsies showed accumulation of PAS-positive, diastase-resistant storage material in both patients and increased lysosomal enzyme activity in patient 1. GBE activity in muscle was negligible in both patients, and Western blot showed decreased GBE protein. One patient had a large homozygous deletion that spanned 627 bp and included exons 8 to 12. The other infant had 2 single base pair deletions, one in exon 10 (1248delT) and the other in exon 12 (1477delC), and each parent was heterozygous for one of the deletions. The large deletion and frame shift mutations seen in these neonatal cases correlates with the severity and earlier onset of symptoms compared with the other forms of GSD IV. The neonatal form of GSD IV is considered extremely rare, but our encountering two patients in close succession suggests that the disease may be underdiagnosed. This diagnosis should be considered in neonates presenting with severe hypotonia and respiratory distress.

**Toward the Development of Genetic System to Study Propionate Metabolic Disorders in *C. Elegans*. C.**

*Venditti*<sup>1,2</sup>, *R. Howard*<sup>1</sup>, *N. Wehrli*<sup>1</sup>, *R. Chandler*<sup>1</sup>, *R. Deering*<sup>1</sup>, *M. Tsai*<sup>1</sup>, *E. Daikhin*<sup>2</sup>, *M. Yudkoff*<sup>2</sup>, *M. Sundaram*<sup>1</sup>, *H. Kazazian Jr*<sup>1</sup>. 1) Dept of Genetics, University of Pennsylvania, Philadelphia, PA; 2) Childrens Hospital of Philadelphia, Philadelphia, PA.

The model organism *C. elegans* has been utilized to study propionate metabolic disorders, specifically methylmalonic acidemia. Database analysis was used to identify homologues of the propionyl CoA carboxylase A (PCCA) and B subunits (PCCB), methylmalonyl CoA racemase (MMCR), MMAA, MMAB, and methylmalonyl CoA mutase (MMCM) genes. RNAi experiments have been reported for some of these genes but have been inconsistent with respect to the nature of the RNA used to target interference. To more thoroughly examine RNAi-effects mediated by bacterial feeding and to explore the potential of this method to induce *C. elegans* mutant phenotypes, we cloned full length cDNAs for each gene, constructed feeding strains, performed RNAi in the presence of proximal and distal metabolites, and scored phenotypes by inspection and chemical analysis. Metabolite measurements of MMA and succinate were performed with GC/MS. Baseline studies showed that wild-type *C. elegans* as well as a variety of anonymous mutant strains produced low levels of MMA under normal laboratory growth conditions. Our initial experiments using MMCM-directed interference revealed that substrate loading with propionate did not disproportionately increase MMA production or sensitivity to exogenous MMA. A MMCR deletion mutant was also studied. While the mutant did not produce an increased amount of MMA under basal conditions, it exhibited increased sensitivity to exogenous MMA and propionate and displayed a low-penetrant post-embryonic phenotype. The results to date indicate that *C. elegans* possesses the full complement of genes necessary to convert propionate into succinate, can produce MMA and has the potential to display a tractable phenotype that could be studied using forward, reverse and chemical genetic methods.

**Single mitochondrial DNA deletion in identical twin brothers.** *E.L. Blakely, R.W. Taylor, L. He, P.F. Chinnery, R.N. Lightowers, A.M. Schaefer, D.M. Turnbull.* Department of Neurology, University of Newcastle upon Tyne, Newcastle upon Tyne, NE2 4HH, United Kingdom.

Defects of mitochondrial DNA (mtDNA) are an important cause of genetic disease in humans. The defect often takes the form of a large-scale rearrangement or point mutation, with clinical features ranging from severe neonatal illness to mild muscle weakness later in life. A recent epidemiology study has shown that single, large-scale mtDNA deletions account for approximately 25% of adult patients with mtDNA disease and are associated with three major clinical syndromes. These are Pearsons syndrome (PS), a severe systemic illness of childhood associated with sideroblastic anaemia; Kearns Sayre syndrome (KSS) which is also associated with multiple system involvement; and chronic progressive external ophthalmoplegia (CPEO) in which the predominant clinical problem relates to an eye movement disturbance and ptosis. In both PS and KSS, the mtDNA deletion may show a wide-spread tissue distribution, whereas in CPEO, the mtDNA deletion is almost exclusively limited to skeletal muscle.

The overwhelming majority of reported cases harboring single mtDNA deletions are sporadic, although the stage in development at which the mtDNA deletion occurs has not fully been established. We have had the unique opportunity to study identical twin brothers, one of whom presented with CPEO and clear histochemical abnormalities indicative of a mtDNA disorder. Southern blot analysis showed that the affected twin harboured high levels of a single 4,115bp mtDNA deletion in his muscle, which was not evident in muscle from the asymptomatic brother. Using sensitive PCR based techniques however, we have also shown that the same mtDNA deletion is present in the muscle from the unaffected twin at very low levels (0.1%), suggesting that the mtDNA deletion was present in the oocyte prior to the development of the embryo.

**CYTOCHROME C OXIDASE ASSEMBLY IN HEALTH AND DISEASE: THE YEAST MODEL. A.**

*Barrientos*<sup>1</sup>, *A. Tzagoloff*<sup>2</sup>. 1) Department of Neurology, John Macdonald Foundation Center for Medical Genetics, University of Miami, Miami, FL; 2) Department of Biological Sciences. Columbia University, New York, NY.

Cytochrome c oxidase (COX) deficiency is the most frequent cause of mitochondrial neuromyopathies in humans. Patients afflicted with these diseases present heterogeneous clinical phenotypes, including Leigh syndrome, muscle weakness and encephalomyopathy. A complete understanding of COX biogenesis is essential for elucidating the molecular basis underlying this group of diseases. The main objective of our work is to use the yeast *Saccharomyces cerevisiae* as a model to investigate COX assembly in wild type cells and in cells with mutations in evolutionary conserved assembly factors. We have recently reported that Shy1p, the yeast homologue of human Surf1p, responsible for most cases of Leigh's syndrome, catalyzes the formation of a COX assembly intermediate involving Cox1p, a mitochondrially encoded catalytic subunit of COX. More recent evidence indicates that this intermediate regulates Cox1p expression in a process involving other COX metabolism factors. Most COX assembly mutants, including *shy1* mutants, exhibit greatly reduced rates of synthesis of Cox1p but not of Cox2p and Cox3p. This phenotype can be explained in, *mss51*, *pet309*, and *oxa1* mutants based on the known functions of their encoded proteins. Both Mss51p and Pet309p are required for translation of *COX1* mRNA while Oxa1p facilitates membrane insertion of Cox1p, which is highly susceptible to proteolysis under these circumstances. The only exception are *cox14* mutants that show normal rates of Cox1p synthesis when tested by pulse labeling. This is also true of strains carrying a *cox14* null mutation combined with mutations in other COX-specific genes. The dominant effect of *cox14* mutations on the rate of Cox1p synthesis suggests a role of Cox14p in regulation of Cox1p expression. An attractive hypothesis consistent with all the available evidence is that Shy1p may regulate Cox1p synthesis by promoting an assembly step which results in the release of Mss51p from an assembly intermediate (formed by the action of Cox14p) thereby making it available for activation of Cox1p translation. *AB is supported by an MDA grant.*

**Increased markers of oxidative damage in a family with the mtA8344G mutation but lacking the classic MERRF phenotype.** *J. Canter, A. Eshaghian, J. Fessel, M. Summar, J. Roberts, J. Morrow, J.E. Sligh, J.L. Haines.* Vanderbilt University Medical Center, Nashville, TN.

Oxygen-derived free radicals generated in mitochondria attack esterified arachidonate in cell membranes, creating prostaglandin-like compounds, such as F2-isoprostanes. Levels of F2-isoprostanes are increased in a variety of degenerative diseases. Isofurans are products of a newly discovered pathway of free radical catalyzed lipid peroxidation that may better reflect oxidative injury at high oxygen tension. We identified the mtA8344G mutation in multiple generations of a large family that demonstrated significant variability in phenotype. Diagnostic evaluation of the 50-year-old proband with olivopontocerebellar degeneration revealed 95% heteroplasmy for the mtA8344G mutation in a muscle biopsy specimen. He has never had seizures and the muscle biopsy failed to reveal ragged red fibers. The proband's maternal grandmother had 5 sisters. We detected the mtA8344G mutation in the only remaining living member of this sibship and in descendants of each of these women. The mtA8344G mutation has been found in 19 adults in this family. Two family members in whom this mutation was undetectable in blood, including the proband's mother, had the mtA8344G mutation in buccal specimens. Symptoms of myoclonus, muscle weakness, ataxia, or hearing loss are reported by 53% of family members with the mutation. However, no family member with both myoclonic epilepsy and progressive myopathy has been identified. The mean F2-isoprostane level in this family (N=22) was 5840 pg/ml compared to controls, 356 pg/ml ( $p < 0.01$ ). No significant difference in F2-isoprostanes levels could be demonstrated within this family on the basis of presence of the mutation or neurological symptoms, but detection may be limited by small sample size. The mean isofuran level for this family was 12766 pg/ml compared to controls of 7010 pg/ml ( $p < 0.01$ ). The degree of heteroplasmy for mtA8344G from buccal specimens correlated strongly with isofuran levels,  $r = 0.82$  ( $p < 0.01$ ). This family with the mtA8344G mutation demonstrates that oxidative injury is present in a wide range of phenotypes, including currently asymptomatic adults.

**De novo mutations in the mitochondrial ND3 gene as a cause of infantile mitochondrial encephalopathy and complex I deficiency.** *R. McFarland<sup>1</sup>, D.M. Kirby<sup>2,3,5</sup>, K.J. Fowler<sup>2,5</sup>, A. Ohtake<sup>4</sup>, M.T. Ryan<sup>4</sup>, D.J. Amor<sup>2,3,5</sup>, J.M. Fletcher<sup>6</sup>, J.W. Dixon<sup>7</sup>, F.A. Collins<sup>8</sup>, D.M. Turnbull<sup>1</sup>, R.W. Taylor<sup>1</sup>, D.R. Thorburn<sup>2,3,5</sup>.* 1) Dept. of Neurology, Neurobiology and Psychiatry, University of Newcastle Upon Tyne, United Kingdom; 2) Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, Australia; 3) Genetic Health Services Victoria, Royal Children's Hospital, Melbourne, Australia; 4) Dept. of Biochemistry, La Trobe University, Melbourne, Australia; 5) Dept. of Paediatrics, University of Melbourne, Australia; 6) Dept. of Chemical Pathology, Womens and Childrens Hospital, Adelaide, Australia; 7) Central Regional Genetic Service, Wellington Hospital, New Zealand; 8) Dept. of Medical Genetics, Childrens Hospital at Westmead, Sydney, Australia.

Energy generation disorders may be caused by mutations in either nuclear or mitochondrial DNA (mtDNA). Respiratory chain complex I deficiency is the commonest energy generation disorder and a frequent cause of Leigh disease and infantile mitochondrial encephalopathies. Most cases of complex I deficiency have been assumed to be caused by nuclear gene defects, but recent reports of mtDNA mutations in patients with Leigh disease and complex I deficiency together with the work we present here, challenge that assumption. We report the first four cases of infantile mitochondrial encephalopathies caused by mutations in the ND3 subunit gene. Three unrelated children have the same novel heteroplasmic mutation (T10158C), only the second mutation reported in ND3, and one has the previously identified T10191C mutation at near-homoplasmic levels. Both mutations cause a much greater reduction in enzyme activity than in the amount of fully assembled complex I, suggesting the ND3 subunit plays an unknown but important role in electron transport. Three cases appear to have a de novo mutation, with no mutation detected in maternal relatives. Our findings indicate that mitochondrial DNA disease may be considerably more prevalent in the pediatric population than currently predicted, and should be considered in patients with infantile mitochondrial encephalopathies and complex I deficiency.

**High dose Coenzyme Q-10 in the treatment of MELAS syndrome.** *LH. Ngu<sup>1</sup>, YS. Choy<sup>1</sup>, S.K. Tan<sup>2</sup>, PKC. Lim<sup>2</sup>, AK. Raihana<sup>3</sup>, LC. Ong<sup>4</sup>.* 1) Genetic/Metabolism Unit, Pediatric Institute, Kuala Lumpur Hospital, Malaysia; 2) Molecular Pathology Unit, Institute of Medical Research; 3) Neurology Institute, Kuala Lumpur Hospital; 4) National University Hospital, Malaysia.

MELAS syndrome is a progressive neurodegenerative disorder with high morbidity and mortality. Coenzyme Q-10, an essential electron transporter and anti-oxidant, has been widely used to treat the condition. Reports of clinical improvement have been documented in some patients using the recommended dosages of 4 to 10 mg/kg/day. However, many patients deteriorated while taking the dosages recommended. We have followed up 12 patients with MELAS syndrome (10 with 3243 mutation), aged 4 months to 42 years, for the past 4 years. 3 patients identified by family screening remained asymptomatic and healthy taking 4mg/kg/day of Coenzyme Q-10, vitamin C 100mg daily and riboflavin 100mg daily. All other 9 patients had more than 2 episodes of crisis with severe lactic acidosis and stroke-like episodes. All were initially prescribed the maximum 10mg/kg/day of coenzyme Q-10, riboflavin 100mg daily, thiamin 100mg daily, vitamin C 100mg daily for children and 200mg tds for adult, vitamin E 400i.u. daily and L-carnitine 50 to 100mg/kg/day. 7 of the 9 patients (78%) had recurrence of the stroke while taking the above mentioned medications. Two passed away without any changes of the medications made during crisis. 5 others had Coenzyme Q-10 increased to 20mg/kg/day in 2 to 3 divided doses for 2 weeks without any clinical or biochemical side effects. The dosage of vitamin C was doubled. All five patients recovered from the stroke-like episodes and blindness faster with less cognitive regression compared to no changes made in Coenzyme Q-10 during earlier crisis. Two patients have been given maintenance Coenzyme Q-10 15mg/kg/day for more than a year without any side effects. But one of them had a relapse. One patient had total blindness for 3 months but his vision recovered with 20mg/kg/day of Coenzyme Q-10. In short, high dose coenzyme Q-10 is save and helpful during crisis of MELAS syndrome. Larger multi-center study is needed for a better evidence of long-term usage of high dose Coenzyme Q-10 in patients with MELAS.

**MNGIE with lack of skeletal muscle involvement and a novel TP splice-site mutation.** *K. Szigeti<sup>1</sup>, L.C. Wong<sup>2</sup>, C. Perng<sup>2</sup>, G.M. Saiji<sup>1</sup>, K. Eldin<sup>1</sup>, A.M. Adesina<sup>1</sup>, D.L. Cass<sup>1</sup>, M. Hirano<sup>3</sup>, J.R. Lupski<sup>1</sup>, F. Scaglia<sup>1</sup>.* 1) Molecular and Human Genetics, Pediatrics, Pathology, Surgery, Baylor College of Medicine, Houston, TX; 2) Institute for Molecular and Human Genetics, Georgetown University, Washington, DC; 3) Department of Neurology, Columbia University College of Physicians and Surgeons, New York, NY.

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive multisystemic disorder caused by thymidine phosphorylase deficiency. Clinically it manifests as severe gastrointestinal dysmotility leading to cachexia; ptosis; external ophthalmoparesis; peripheral neuropathy and leukoencephalopathy. We report a patient with a classical MNGIE clinical presentation, but surprisingly, without skeletal muscle involvement at the morphological, enzymatic and mitochondrial DNA levels. MNGIE was diagnosed by markedly elevated plasma thymidine level and reduced thymidine phosphorylase activity. Molecular genetic analysis revealed a homozygous novel splice site mutation in the thymidine phosphorylase gene (TP). Sequencing of the coding regions and the adjacent splicing junctions revealed the presence of a novel homozygous GC transversion at the splice acceptor site of the second coding exon. RT-PCR analysis followed by sequencing of the expected product revealed that the second coding exon is skipped and an alternative splice donor site becomes activated. The alternative splicing results in deletion of 234 nucleotides from the mRNA sequence that by conceptual translation leads to an in-frame deletion of 78 amino acids from the protein. The deleted fragment contains the catalytic His116 residue, thus its absence likely impairs function. The predicted loss-of-function was confirmed by the markedly reduced enzyme activity measured in peripheral blood. Interestingly, since the deletion is in-frame, the protein preserved its antigenicity to the TP antibody. Immunohistochemistry revealed marked TP expression in the CNS in contradistinction to what has been observed in rodents. We emphasize the necessity to examine the most significantly affected tissue in order to arrive at an accurate diagnosis.

**Tissue, bile and blood carnitine/acylcarnitine profiles during exercise and a cold challenge with fasting in VLCAD knockout mice.** *U. Spiekerkoetter*<sup>1,2,3</sup>, *C. Tokunaga*<sup>2</sup>, *U. Wendel*<sup>1</sup>, *E. Mayatepek*<sup>1</sup>, *F.M. Vaz*<sup>3</sup>, *N. van Vlies*<sup>3</sup>, *J.P.N. Ruiten*<sup>3</sup>, *M. Duran*<sup>3</sup>, *L. Ijlst*<sup>3</sup>, *F.A. Wijburg*<sup>3</sup>, *R.J.A. Wanders*<sup>3</sup>, *A.W. Strauss*<sup>2</sup>. 1) Department of General Pediatrics, University Children's Hospital, Duesseldorf, Germany; 2) Department of Pediatrics, Vanderbilt Univ, Nashville, TN; 3) Academic Medical Center, Amsterdam, The Netherlands.

In human very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, clinical symptoms are induced by external stressors with involvement of heart, liver and skeletal muscle (SM). The VLCAD KO mouse exhibits stress-induced hypoglycemia, skeletal myopathy and cold intolerance, symptoms resembling human VLCADD, and is a valuable model to study the effects of exercise and a cold challenge with fasting, conditions triggering disease manifestation. We characterized the biochemical phenotype in response to these stressors. Carnitine/acylcarnitine profiles were measured by tandem mass spectrometry using methanol extraction for blood and bile samples and acetonitrile extraction for tissues. During fasting-induced lipolysis, blood C16-C18 acylcarnitines increased 2 to 3-fold in KO mice, more than after exercise. In bile, C16-C18 acylcarnitines were 5 to 10-fold higher than in blood and strongly increased after fasting; bile-blood ratios remained constant. In liver, a 2-fold increase of C16-C18 acylcarnitines was observed after the cold challenge with fasting as reflected by bile and blood profiles. After exercise, liver acylcarnitines did not increase significantly compared to unstressed conditions despite an increase in blood. These acylcarnitines likely derived from production in SM with a 2 to 3-fold increase after exercise. Whereas blood free carnitine significantly decreased after the cold challenge with fasting, liver concentrations remained stable. Exercise resulted in a significant decrease of free carnitine in SM. In conclusion, blood acylcarnitine profiles reflect the overall production of acylcarnitines in various tissues. In response to different stressors, specific organs are affected and acylcarnitine production appears to be tissue-specific. Free carnitine concentrations also decrease tissue-specifically due to different stressors.

**Evaluation of the Mitochondrial Disease Criteria (MDC) scoring system in the South African population. I.**

*Smuts<sup>1</sup>, A.C. Van Brummelen<sup>2</sup>, D.C. Wallace<sup>3</sup>, A. Olckers<sup>2,4</sup>.* 1) Paediatrics, University of Pretoria, Pretoria, South Africa; 2) Centre for Genome Research, Potchefstroom University for CHE, Pretoria, South Africa; 3) Centre for Molecular and Mitochondrial Medicine and Genetics, Univ of California at Irvine, Irvine, USA; 4) DNAbiotec (Pty) Ltd, Pretoria, South Africa.

The clinical diagnosis of mitochondrial disorders is complex and challenging. For this reason, Wolf et al. (2002) proposed consensus diagnostic criteria, the Mitochondrial Disease Criteria (MDC), in order to standardise the clinical diagnosis of this group of clinically heterogeneous disorders. The MDC relies on the following systems being predominantly affected: muscle, central nervous system and multi-system involvement. To date, we have scored 65 subjects according to the proposed MDC scoring system. 27 of our patients we scored to be probable mitochondrial disease, with an MDC score of between 5-7. We assessed eight of our patients to be in the definite mitochondrial disease class, with an MDC score of 8 and higher. Given that muscle biopsies are difficult and sometimes not practical in our environment, the maximum score that we could generate for any of our patients was 8. The four additional points of the MDC score would have been generated via data from a muscle biopsy. The above data demonstrates that the MDC scoring system is able to discriminate between our patient group, based on clinical and biochemical data alone. Genetic studies are underway to elucidate the genotypes responsible for the phenotypes segregating in the above patients.

**Molecular screening of patients with mitochondrial disorders in the South African population.** *A.C. Van Brummelen<sup>1</sup>, I. Smuts<sup>2</sup>, D.C. Wallace<sup>3</sup>, A. Olckers<sup>1,4</sup>.* 1) Centre for Genome Research, Potchefstroom University, Pretoria, South Africa; 2) Dept of Paediatrics, Univ of Pretoria, South Africa; 3) Centre for Molecular and Mitochondrial Medicine and Genetics, Univ of California at Irvine, Irvine, USA; 4) DNAbiotec (Pty) Ltd, Pretoria, South Africa.

In a previous study, gDNA of 53 patients with suspected mitochondrial disorders were screened for 25 reported mtDNA mutations via an RFLP, and subsequently a sequencing strategy encompassing the 3000, 8000 and 9000 regions. The above samples were screened from DNA collected via whole blood and fibroblast cultures. Only one reported mitochondrial mutation (A3243G), associated with MELAS, was detected in a single proband and subsequently in her extended family with different levels of heteroplasmy. However, overall, six novel alterations were also detected. These results raised the question whether the genetic aetiology of mitochondrial disorders in our population was indeed similar to those reported elsewhere, since only one reported mutation was detected in this extensive study. The above study was extended to investigate whether the novel alterations were also present in other South African patients. To this end, an additional subset of 22 well characterised (clinically and biochemically) patients were included, for a total of 75 patients. They were screened via single stranded sequencing of the tRNA<sup>Leu</sup>(UUR), tRNA<sup>Lys</sup> and ATPase 6 mitochondrial genes, for the reported as well as the above six novel alterations.

**Transcriptional responses in nuclear encoded human mitochondrial complex I deficiency.** *F.H. van der Westhuizen*<sup>1,2</sup>, *J. Smeitink*<sup>2</sup>, *L.P. van den Heuvel*<sup>2</sup>, *R. Smeets*<sup>2</sup>, *J.A. Veltman*<sup>3</sup>, *R. Pfundt*<sup>3</sup>, *A. Geurts van Kessel*<sup>3</sup>, *B.M. Ursing*<sup>4</sup>. 1) School for Chemistry and Biochemistry, PUforCHE, Potchefstroom, South Africa; 2) Nijmegen Center for Mitochondrial Disorders, UMC Nijmegen, The Netherlands; 3) Department of Human Genetics, UMC Nijmegen, The Netherlands; 4) Center for Genomics and Bioinformatics, Karolinska Institute, Sweden.

NADH:ubiquinone oxidoreductase (complex I) deficiency is one of the most frequently encountered defects of the mitochondrial energy generating system. A deficient activity of this multi-protein oxidative phosphorylation system enzyme complex can be caused by mutations in the mitochondrial or the nuclear genome and leads to a remarkably wide variety in clinical disease expression. We investigated the transcriptional responses by microarray in fibroblast cell lines harboring nuclear DNA encoded mutations in the NDUFS2, NDUFS4, NDUFS7, NDUFS8, and NDUFV1 subunits of complex I. A microarray containing a selection of approximately 600 genes involved in bioenergetics and other mitochondria-related functions were constructed and evaluated in a novel experimental approach. Transcriptional responses of control and patient fibroblasts were compared by changing the cell culture conditions from glycolytic to oxidative metabolism. Approximately 60 genes displayed differential expression under these conditions in either all mutated cell lines or selected cell lines only. A marked induction of all investigated metallothioneins as well as ATP1G1 transcripts was detected in all patient cell lines. In addition, individual transcriptional responses such as induction of heat shock protein transcripts, decreased PDK1, BNIP3 and mitochondrial genome encoding gene transcripts only occurred in selected patient cell lines. The observed transcript profile in all complex I mutated cell lines pointed to a common, putative defensive response related to oxidative stress. With the exception of one cell line, patient cell lines could be grouped using hierarchical clustering. Although further investigations is warranted, these results underline the great potential of functional genomics to gain new insights into inborn errors of energy metabolism.

**A Yeast Model for McCune-Albright Syndrome.** *R. Pals-Rylaarsdam, M. Koster, J. Mitchell.* Biology, Trinity Christian College, Palos Heights, IL.

Patients with McCune-Albright Syndrome (MAS) suffer from a variety of complications due to the post-zygotic mutation of the alpha subunit of the stimulatory G-protein  $G_s$ . This mutation causes signal-independent activity of the G-protein leading to high levels of cAMP in the affected cells. Patients experience a variety of conditions such as the abnormal development of bone tissue, known as polyostotic fibrous dysplasia, as well as other abnormalities including precocious puberty, ovarian cyst formation, and skin hyperpigmentation. We have developed a model system to study the effects of MAS mutations using an engineered strain of the budding yeast *Saccharomyces cerevisiae*. Haploid yeast identify mating partners by responding to pheromones sensed by G-protein coupled receptors. The downstream signaling pathways are well characterized and homologous to human G-protein signaling pathways. Activation of the yeast pheromone response inhibits cell division; thus an active G-protein prevents colony formation. One normal copy of the G-protein gene, even in the presence of an activated copy of the gene, is sufficient to allow growth. We introduced the MAS mutation into the analogous site in the yeast G subunit, and identified active and inactive mutants in the G-protein based on the ability of the yeast to form colonies on 5-FOA medium, an environment that selected for loss of the normal copy of the G-protein gene. We replaced a section of the yeast G gene with randomly mutated DNA sequences containing approximately five mutations each. A library of 1600 plasmids carrying random mutations was screened in the yeast model system for suppression of the McCune-Albright mutation. From 1046 colonies, four plasmids encoding a G-protein with a corrected phenotype were isolated. The successful identification of these intragenic suppressors may lead to structural prediction of small molecules capable of regulating the defective G-proteins.

**Biotin deficiency in the rat as a model of the genetic expression of inter-organ mammalian carboxylation.** A. Velazquez, N. Rodriguez-Fuentes, S. Cano-Colin. Nutritional Genetics, Natl Univ Mexico, Mexico, D F, Mexico.

Biotin-dependent carboxylation reactions are very important in the metabolism of carbohydrates, lipids and proteins. Their genetic expression rely on processes whose Mendelian defects result in Multiple Carboxylase Deficiency (MCD), its phenocopy being biotin deficiency (BD). To better understand the molecular pathophysiology of MCD we studied the effects of BD on rats liver, adipose tissue, heart, ileum and spleen. There were wide differences among these organs: adipose had the greatest decrease in pyruvate (PC) and propionyl CoA (PCC) carboxylase activities and protein amounts; heart had the lesser decrease; ileum was very deficient in PCC but not in PC. There was no detectable PC activity or mass in control and BD spleen, and PCC was very deficient in this BD organ. To look for an explanation of these results, we measured their mRNAs and the Na-dependent multivitamin transporter (SMVT). PC and PCC mRNAs diminished in ileum and spleen (there was detectable PC mRNA in spleen), but they did not change in liver, adipose and heart of BD animals. The SMVT mRNA increased in BD liver and adipose; decreased in ileum, and did not change in heart and spleen. Therefore, the observed carboxylation changes do not seem to be related to alterations in biotin transport or carboxylases transcription. An additional observation was a very different distribution of carboxylase activities and masses among different organs in the control animals, indicating their differential genetic expression most likely in accordance with the distinct physiological roles of these organs. This investigation complements our previous work on kidney, muscle and brain (J Nutr 2001, 131:1909).

Program Nr: 1719 from 2003 ASHG Annual Meeting

**A PCR/OLA assay for reflex testing of I506V, I507V and C508 mutations in the human CFTR gene.** *E. Swartzman, S. Koepf, L. Nguyen, E. Schreiber.* Celera Diagnostics, Alameda, CA.

Cystic fibrosis (CF) is an autosomal recessive disorder that is caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR). CF is the most common lethal genetic disease in the US and the American College of Medical Genetics (ACMG) has recommended population-wide, prenatal CF carrier screening. Among the greater than 1000 known mutations, the deletion F508 mutation is the most common and accounts for 60-80% of the CF mutant alleles in certain populations. We have previously developed a single tube PCR/OLA assay for research use to detect 32 common mutations and alleles including the 25 mutations recommended by the ACMG. Here we describe a revised exon 10 reflex assay that is utilized only to verify a homozygous deletion of the F508 or I507 codon and exclude a potential false positive result due to interference by the single nucleotide polymorphisms (SNPs) of V506, V507 or C508. The reflex assay consists of a PCR reaction to amplify exon 10 of the CFTR gene followed by an oligo ligation assay (OLA) using fluorescently labeled probes to detect either normal or mutant CF alleles. The allele specific fragments are subsequently analyzed by electrophoretic sequence coded separation (SCS) using the ABI PRISM 3100 Genetic Analyzer. The normal or mutant genotypes are then automatically identified using GenoTyper or GeneMapper software. The performance of the assay was evaluated using mutant genomic and synthetic DNA templates and the expected results were obtained.

**Neurodevelopmental pattern of succinic semialdehyde dehydrogenase deficiency (4-hydroxybutiric aciduria).** A. PHILIPPE<sup>1</sup>, J. DERON<sup>1</sup>, P. DE LONLAY<sup>1</sup>, K.M. GIBSON<sup>2</sup>, D. RABIER<sup>3</sup>, A. MUNNICH<sup>1</sup>. 1) INSERM U 393, Hopital Necker-Enfants Malades, PARIS, France; 2) Biochemical Genetics Laboratory, Oregon Health Sciences University, Portland,USA; 3) Laboratoire de Biochimie Medicale B,Hopital Necker-Enfants Malades, Paris,.

Succinic semialdehyde dehydrogenase (SSADH Mc Kusick 271980) deficiency is a defect in gamma-aminobutyric acid degradation, resulting in the accumulation of gamma-hydroxybutyric acid (GHB) and causing neurological and cognitive disorders of varying severity. Their non-specific nature and difficulties in detection of urinary GHB explain why this disorder is largely underdiagnosed. At least 150 affected individuals have been hitherto identified but only three adults have been reported. Here, we described two additional cases in adult brothers and emphasize the remarkable similarity of their course from birth into adulthood. Early assessment revealed that their delayed language was due to a verbal agnosia. A review of their early motor development revealed that both sibs presented hypotonia from birth and mild delay of motor milestones. Both sibs also had a broad-based unsteady gait that spontaneously improved at 7 years. During or shortly after adolescence, both experienced hallucinations. Moreover, the elder brother had sleep disturbances since early childhood and some years ago, he experienced daytime attacks of sleep triggered by emotion of delight, which led to the investigation of a possible diagnosis of narcolepsy before SSADH deficiency was recognised. Finally, cerebral MRI in both brothers revealed a moderate cerebellar vermis atrophy and a retrocerebellar cyst and in the younger brother, cerebral MRI also detected an increased T2 signal intensity in the anterior part of the external globus pallidus. We discuss the main clinical features of SSADH deficiency in relation to physiopathologic involvement of GHB and try to identify the most specific neurodevelopmental pattern of this disorder.

**Transport of methylmalonic acid by organic anion transporters.** X. Fu<sup>1</sup>, T.J. Urban<sup>2</sup>, K.M. Giacomini<sup>2</sup>, S. Packman<sup>1</sup>. 1) Dept of Pediatrics, Div of Medical Genetics; 2) Dept of Biopharmaceutical Science, Univ of California San Francisco, CA.

The mechanisms of neurotoxicity in methylmalonic acidemia and other heritable organic acidemias are not understood. For example, in the methylmalonic acidemias of diverse etiologies, therapies that reduce the concentration of methylmalonic acid (MMA) and related metabolites in the systemic circulation and peripheral tissues, do not uniformly ameliorate or arrest progressive neurologic deterioration. It is therefore possible that central nervous system and cerebrospinal fluid (CSF) accumulation of MMA might continue unabated, even in the face of peripheral tissue reductions of metabolite concentrations. To this end, we have begun to ask how MMA is transported into and out of the CSF. As a first step towards characterization of choroid plexus (CP) transport of MMA, we characterized MMA transport by two different and specific organic anion transporters known to be expressed in CP, using a *X. laevis* oocyte expression system. Capped cRNAs encoding the rat sodium-dependent dicarboxylate transporter (rNaDC1) and the human organic anion transporter (hOAT1) were transcribed *in vitro* and injected into mature oocytes, the uptake of <sup>14</sup>C-MMA and prototypic substrates (<sup>3</sup>H-succinate (rNaDC1) and <sup>3</sup>H-p-aminohippurate (hOAT1)) was determined. <sup>14</sup>C-MMA was taken up by both rNaDC1 (41±2.95 pmol/h/oocyte vs. 17±1.22 for the control, p<0.001) and hOAT1 (40±4.13 pmol/h/oocyte vs. 14±1.44 for the control, p<0.001). In addition, the uptake of <sup>3</sup>H-succinate into oocytes expressing rNaDC1 was inhibited 24% by 5 mM MMA (p<0.001), and the uptake of <sup>3</sup>H-aminohippurate into oocytes expressing hOAT1 was inhibited 56% by 5 mM MMA (p<0.001). We conclude that MMA is a low-affinity substrate and inhibitor of both rNaDC1 and hOAT1, in this expression system. The low potency of interaction of MMA with these CP transporters is consistent with the finding that MMA accumulates in the CSF of patients treated for methylmalonic acidemia. We are further characterizing the kinetics and specificity of transport of MMA by these organic anion transporters, and determining their localization in CP epithelial cells.

**A mild variant of Canavan disease with a novel mutation in ASPA gene.** *Y. Anikster<sup>1</sup>, G.S. Salomons<sup>2</sup>, S.H. Korman<sup>3</sup>, V. Barash<sup>3</sup>, N. Gal<sup>1</sup>, J. Zlotnik<sup>1</sup>, T. Masos<sup>1</sup>, B. Ben-Zeev<sup>4</sup>.* 1) Metabolic Disease Unit, Safra Children Hospital, Tel-Hashomer; 2) VU University Medical Center, Amsterdam, The Netherlands; 3) Clinical Biochemistry, Hadassah University Hospital, Jerusalem; 4) Pediatric Neurology, Safra Children Hospital, Tel-Hashomer, Israel.

Canavan disease (CD) is a neurodegenerative disorder caused by mutation in the aspartoacylase (ASPA) gene. The classic phenotype is of severe neurological impairment noticed before 6 months of age, followed by rapidly progressive mental retardation. Life expectancy is usually into the teens, and optic atrophy is typical. We present a mild variant in a 20 year old male descendant of non-consanguineous Libyan-Jewish parents. At 3 months he presented with hypotonia, macrocephaly and nystagmus. However, in the following years he showed developmental progress, was able to walk with a walker from the age of 4, could talk in sentences although with accompanied dysarthria, became toilet trained and had significant hand usage (could draw and feed himself). He was assessed as functioning in the moderate mental retardation range at the age of 5. He never showed any visual impairment and has normal looking optic discs. In his second decade, aggravation of ataxia and spasticity resulted in loss of ambulation, and he became wheelchair bound, but was still alert and interactive. CT scan followed by MRI showed progressive cerebral and cerebellar atrophy and abnormal diffuse white matter signal in cerebrum, midbrain, cerebellum and basal ganglia. Despite normal metabolic screen in the past, urinary organic acids were repeated and elevated NAA was detected. We confirmed the diagnosis by aspartoacylase assay in fibroblasts. By sequencing all the ASPA exons, we found a novel missense mutation. The transversion of G to C at position 610 resulted in one amino acid change the aspartic acid 204 (which is conserved in evolution) to histidine. The mutation creates *EcoRI* restriction site and allows us to show segregation in the family. In screening 174 anonymous Libyan-Jews, we found no allele which harbored this mutation. Our results allow prenatal diagnosis and carrier detection in this family and widen the clinical spectrum of CD.

**A pilot study of nitisinone in Alkaptonuria.** *P. Suwannarat*<sup>1</sup>, *I. Bernardini*<sup>1</sup>, *M. Kaiser-Kupfer*<sup>2</sup>, *E. Tsilou*<sup>2</sup>, *B. Rubin*<sup>2</sup>, *K. O'Brien*<sup>1</sup>, *W. Gahl*<sup>1</sup>. 1) Section on Human Biochemical Genetics, Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 2) Clinical Research Branch, National Eye Institute, National Institutes of Health, Bethesda, MD.

Alkaptonuria (AKU) is an autosomal recessive disorder due to deficiency of the enzyme homogentisic acid dioxygenase in the tyrosine degradation pathway. AKU is characterized by daily excretion of gram quantities of urinary homogentisic acid (HGA), vertebral disk collapse, arthritis, joint destruction requiring replacement, and ochronosis, or dark pigmentation of tissues due to HGA and its oxidation products. No effective therapy exists. However, 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexadine (nitisinone) inhibits 4-hydroxyphenylpyruvate dioxygenase, the enzyme that produces HGA. We treated 3 patients with nitisinone, titrating the dose to lower HGA excretion to less than 0.5 gm/day. The doses were 0.35 mg/day (divided bid) for one week and then 1.05 mg/day for 3 months. Initial HGA levels of 3.4, 3.7, and 2.6 gm/day fell to ~80-300 mg/day. Plasma tyrosine concentrations, initially ~60 micromolar, rose to ~1100 micromolar. Weekly eye examinations revealed no signs of corneal toxicity. After 5 weeks of treatment, patient #1 passed several kidney stones but remained on study. After 11 weeks, patient #3, who previously had asymptomatic aortic stenosis, developed symptoms of lightheadedness and dyspnea on exertion. She was removed from the study and received a valve replacement. The other 2 patients completed 12 and 13 weeks of nitisinone therapy. During the last week of treatment, each received only 40 grams of protein per day, and plasma tyrosine and urinary HGA concentrations were monitored. Long term clinical trials are planned to determine the benefit of nitisinone in preventing joint deterioration and pain.

**Rescue of peroxisome assembly at 30C: expanding the type of responsive mutations in *PEX6* defective cells.** *N. Braverman*<sup>1</sup>, *L. Chen*<sup>1</sup>, *S. Dasinger*<sup>1</sup>, *A. Moser*<sup>2</sup>, *H. Moser*<sup>2</sup>, *S. Steinberg*<sup>2</sup>. 1) Inst Genetic Medicine, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Kennedy Krieger Inst, Baltimore, MD.

The peroxisome biogenesis disorders (PBD) are a heterogeneous group of autosomal recessive diseases caused by defects in *PEX* genes. Patient phenotypes range from severe Zellweger syndrome (ZS) to milder neonatal adrenoleukodystrophy (NALD) and infantile Refsum disease (IRD). In general, the milder patients have *PEX* mutations predicted to result in residual peroxin function. Recently, several groups have reported that fibroblasts from NALD-IRD patients show improvement in the import of matrix proteins into peroxisomes when grown at 30C. This temperature sensitive phenotype, thus far associated with missense alleles, is of importance as it might reflect a partially functional, but unstable protein at body temperature that could then be supported by stabilizing drugs.

One of the larger PBD groups is caused by defects in *PEX6*, which encodes a member of the AAA family of ATPases. Only 16 probands with *PEX6* defects are reported to date. We present an analysis of *PEX6* in 20 probands from our collection: 12 ZS, 6 NALD and 2 IRD. We amplified and sequenced all 17 exons and adjacent intronic regions, and identified mutations in 37/40 alleles. The majority were unique. 41% were missense, 51% insertion or deletion alleles and 8% were splice site changes. Surprisingly, several NALD patients had mutations that shift the reading frame on both alleles. To investigate genotype-phenotype correlations, we analyzed *PEX6* transcripts by northern analysis and RT/PCR. We also evaluated import of endogenous PTS1 and PTS2-targeted matrix proteins and peroxisome morphology by indirect immunofluorescence microscopy in fibroblasts cultured at 30 and 37. We found dramatic improvement in these parameters at 30 in 5 patients with NALD-IRD and in 2 of 5 ZS patients. Remarkably, temperature sensitive import was observed in cells from patients with ZS and NALD harboring out of frame transcripts. Taken together, these results expand the current observations on temperature sensitivity and implicate the translation of truncated Pex6 peroxins that are stabilized at 30.

**PEX26 mutations and cellular phenotype of patients with complementation group (CG) 8 peroxisome biogenesis disorder (PBD).** *S. Weller*<sup>1</sup>, *S.J. Gould*<sup>2</sup>, *H.W. Moser*<sup>3</sup>, *D. Valle*<sup>1,4</sup>. 1) Inst Genetic Medicine; 2) Dept of Biological Chemistry; 3) Kennedy Krieger Inst; 4) Howard Hughes Medical Institute, Johns Hopkins Univ, Baltimore, MD.

The Zellweger spectrum subgroup of the PBDs comprises a genetically heterogenous group of autosomal recessive diseases characterized by deficiency of multiple peroxisomal enzymes. Eleven CGs are known. Recently PEX26 was identified as the gene responsible for CG8, the only unsolved CG, with a homozygous missense mutation reported in 1 patient. PEX26 is an integral peroxisomal membrane protein of 305 aa with the N-terminus exposed to the cytosol and seems to be essential for the localization of PEX1-PEX6 complexes to peroxisomes. PEX1 and 6 are AAA ATPases and their function in peroxisome biogenesis is controversial. Here we report 8 unrelated CG8 patients with clinical phenotypes ranging from mild to severe. We identified 9 PEX26 alleles that account for all 16 mutant PEX26 genes in this patient group and include 11 missense and 2 splice site mutations, a single bp insertion, a single bp deletion, and a duplication of 122 coding bp. To determine the functional consequences of these mutations, we characterized the cellular phenotype of the PEX26 deficient patients fibroblasts by transfection and immunofluorescence studies with a series of reporter genes designed to test various aspects of peroxisomal protein import and compared them to cells with null mutations in PEX1, 6 or 5 (CG1,4&2). We observed a range of severity in the cellular phenotype of PEX26 deficient cell lines that corresponds, in part, to the clinical phenotype of the patients. A patient with a homozygous splice site mutation (IVS2+1G>T) shows a pronounced deficiency in peroxisomal matrix protein import and a severe clinical phenotype. A patient homozygous for R98W has a milder clinical phenotype and the mildest import deficiency. A compound heterozygote for R98W and L44P had a much more severe cellular phenotype. None of the CG8 cell lines had an import defect as severe as cell lines with null mutations in PEX1, PEX6, or PEX5. This ongoing analysis suggests a rough correlation between PEX26 genotype, cellular and clinical phenotype.

**Identification of novel polymorphisms in cytochrome P450 genes by amplification and sequencing of 93 human DNA samples.** *J. Solus, R. Harris, B. Arietta, J. Steward, D. Sexton, C. McMunn, P. Ihrle, J. Mehall, T. Edwards, E. Dawson.* Research & Development, BioVentures, Inc, Murfreesboro, TN.

Several cytochrome P450 genes known to be involved in drug metabolism were screened for genetic polymorphisms in 186 human chromosomes by genomic amplification and direct sequencing of the exons and flanking intronic regions. The targets screened were CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 and CYP3A5. Primers were designed and tested to amplify the genomic target without co-amplification of gene family members or pseudogenes. A total of 59 kb of bidirectional sequence data was generated and scored for each chromosome. Of the 93 self-described human DNA samples screened, 36 were Caucasian, 16 African American, 10 Chinese, 10 Japanese, and 10 Southeast Asian. In addition, 11 samples were of anonymous ethnic origin.

The method of direct sequencing identified 416 polymorphic sites in the eleven CYP450 genes screened. There were 398 single base substitutions and 18 insertions or deletions (indels). Of the polymorphic sites, 183 occurred within the wild type exonic portion of the genes, and the remaining 233 were intronic or intergenic. Of the sites within exons, 4 were in the 5UTR, 49 were in the 3UTR, and 120 were in the coding region. Of these, 50 were silent changes in the coding region. Many of these polymorphisms have been previously reported: 22% by the Human Cytochrome P450 Allele Nomenclature Committee and 18% by NCBI dbSNP. 66% of the polymorphisms have not been reported by either of these sources. Of these novel polymorphisms, 98 are within the exons of the genes, 30 are within the coding regions and result in amino acid changes, and the remaining 24 are silent. There are 3 within the 5UTRs and 41 within the 3UTRs. Of the 175 novel changes in the intronic or intergenic portions of the genes, 8 are within 10 bp of an exon-intron boundary and consequently may have an effect upon splicing.

**Oxidant Stress, Polymorphisms in GCLC, and Pulmonary Hypertension in Congenital Heart Surgery.** *K.L. Dyer<sup>1</sup>, A.S. Willis<sup>2</sup>, F.E. Barr<sup>3</sup>, G. Cunningham<sup>4</sup>, J.L. Haines<sup>5</sup>, T.P. Doyle<sup>1</sup>, T.P. Graham<sup>1</sup>, B.W. Christman<sup>4</sup>, M.L. Summar<sup>5,6</sup>.* 1) Pediatric Cardiology, VUMC, Nashville, TN; 2) Molecular Physiology and Biophysics, VUMC, Nashville, TN; 3) Pediatric Critical Care, VUMC, Nashville, TN; 4) Pulmonary Medicine, VUMC, Nashville, TN; 5) Program in Human Genetics, VUMC, Nashville, TN; 6) Pediatric Genetics, VUMC, Nashville, TN.

Pulmonary hypertension is a potentially serious side effect of congenital heart surgery. Ischemia-reperfusion injury, which is associated with cardiopulmonary bypass and mediated by free radical activity, may contribute to the pathogenesis of pulmonary hypertension. Glutamate cysteine ligase catalyzes the first, rate-limiting step in the synthesis of glutathione, an important biomolecule for the scavenging of free radicals. Eleven polymorphisms have been identified in GCLC, the gene that encodes the catalytic subunit of glutamate cysteine ligase. To determine if a relationship between GCLC polymorphisms, markers of oxidative stress, and development of pulmonary hypertension exist, urine isoprostane levels at several timepoints, immediately before and after surgery and at 12, 24, and 48 hours postoperation were measured in 60 children undergoing surgery to repair congenital heart defects. Each patient was also genotyped for 11 polymorphisms in GCLC. One GCLC polymorphism, 1974-1975insCAGC, had a statistically significant association with development of pulmonary hypertension using chi-square analysis ( $p=0.035$ ). Isoprostane levels immediately following surgery were significantly higher in patients who developed pulmonary hypertension. Isoprostane levels were also significantly higher at the same time point in patients homozygous for the inclusion of the 4 bp insertion at the 1974-1975insCAGC locus. These results may indicate that genotype for the GCLC polymorphism 1974-1975insCAGC may be a risk factor or marker for the development of pulmonary hypertension following cardiopulmonary bypass and cardiac surgery.

Program Nr: 1728 from 2003 ASHG Annual Meeting

**Functional Analysis of a Nonsynonymous Polymorphism in Glutamate Cysteine Ligase Catalytic Subunit Gene.**

A.S. Willis<sup>1</sup>, M.L. Freeman<sup>2</sup>, S.R. Konjeti<sup>2</sup>, J.L. Haines<sup>3</sup>, A. Wilson<sup>4</sup>, B. Kennedy<sup>4</sup>, S.M. Williams<sup>3</sup>, M.L. Summar<sup>4</sup>. 1) Molecular Physiology and Biophysics, VUMC, Nashville, TN; 2) Radiation Biology, VUMC, Nashville, TN; 3) Program in Human Genetics, VUMC, Nashville, TN; 4) Pediatric Genetics, VUMC, Nashville, TN.

Glutamate cysteine ligase catalyzes the first, rate-limiting step in the *de novo* synthesis of glutathione, an important tripeptide. One of the most important functions of glutathione is the scavenging of free radicals to prevent oxidative damage, which is thought to play a role in many diseases, such as neurodegeneration and cancer. We have previously identified 11 polymorphisms in GCLC, the gene that encodes the catalytic subunit of glutamate cysteine ligase. One of these polymorphisms, C1384T, is a nonsynonymous polymorphism. The rare allele changes a proline to serine at position 462 and has not been observed in Caucasians. We have undertaken the study of the functional aspects of this polymorphism. Using clones of the coding region of both human GCLC and GCLM, we have expressed both versions of the GCLC polymorphism in bacteria. Western blot demonstrates expression of soluble normal sized GCLC protein. Enzyme activity assays using measurement of production of radioactive inorganic phosphate demonstrates activity above the baseline in bacterial cells. Comparison of the two forms of GCLC demonstrates an increase in activity for the less common allele seen only in African or African-descent individuals. This change in activity may affect the susceptibility of carriers to oxidative damage and will be examined in populations at risk such as sickle cell anemia, Alzheimers, Parkinsons disease, and others.

**A novel mechanism for the control of biotin utilization in human cells is impaired in patients with multiple carboxylase deficiency.** *D. Pacheco-Alvarez<sup>1</sup>, R.S. Solorzano-Vargas<sup>1</sup>, A. Velzquez<sup>1</sup>, R.A. Gravel<sup>2</sup>, A. Leon-Del-Rio<sup>1</sup>.*  
1) Department of Molecular Biology, Universidad Nacional Autonoma de Mexico; 2) Department of Biochemistry University of Calgary, Calgary, Alberta, Canada.

Biotin is a water-soluble vitamin that functions as cofactor of enzymes known as biotin-dependent carboxylases. Biotinylation of carboxylases is catalyzed by holocarboxylase synthetase (HCS). HCS is also involved in the transcriptional regulation of several genes in human cells. To ensure the biotin supply higher organisms have evolved a complex system composed by a Na-dependent vitamin transporter (SMVT), HCS, five carboxylases and biotinidase responsible for biotin recycling. Here we show that the transcription of genes participating in the biotin cycle is regulated by biotin availability. Biotin deficiency result of a low vitamin diet or the disease multiple carboxylase deficiency, down-regulate the expression of HCS, SMVT and PC mRNAs to 10-20 % of the control. Biotinidase mRNA levels are not affected by neither of these conditions. This suggests that biotin deficiency reduces the transport and utilization of dietary biotin while leaving unaffected the ability to recycle endogenous biotin. To explore the metabolic role of this mechanism we developed an animal model for biotin deficiency. Deficient rats showed a 70%-80% reduction in the mRNA levels of HCS, PC, SMVT in liver, kidney and muscle but the brain mRNA levels were not affected. Biotinidase mRNA did not show a significant change in liver, muscle and kidney but increased 20-30% over control values in the brain. We suggest that, during fasting transcriptional downregulation of SMVT, HCS and carboxylases in liver, kidney and muscle allows a continuous supply of biotin to the brain. Endogenous biotin recycling by biotinidase may allow the survival of peripheral tissues and avoiding competition with the brain for low biotin plasma concentrations. The significance of this process may be related to the fact that 25% of the energy used by the brain is supplied via anaplerosis of the citric acid cycle by biotin dependent enzymes piruvate carboxylase (PC) and propionyl-CoA carboxylase (PCC).

**Fatty acid imbalance in cystic fibrosis associated with pulmonary disease severity.** *J. Hoover-Fong<sup>1</sup>, A. Moser<sup>2</sup>, H. Moser<sup>2</sup>, M. Diener-West<sup>3</sup>, K. O'Brien<sup>3</sup>, A. Hamosh<sup>1</sup>, B. Rosenstein<sup>1</sup>, M. Boyle<sup>1</sup>, G. Cutting<sup>1</sup>.* 1) Johns Hopkins Univ, Baltimore, MD; 2) Kennedy-Kreiger Inst, Baltimore, MD; 3) Johns Hopkins School of Public Health, Baltimore, MD.

**Objective:** Studies indicate fatty acids (FA) in plasma, RBCs & tissues of CF patients differ from controls. The relation between FAs & CF disease severity is unclear. We measured many fasting plasma, RBC & nasal epithelial FAs in CF & age-matched controls to determine if: 1) FAs are different, 2) FA imbalance is related to disease severity. We focused on docosahexaenoic (DHA) & arachidonic acid (AA) since AA is an inflammatory precursor & competes with DHA for membrane phospholipid uptake. **Methods:** 36 controls (7-11y; n=9, 12-17y; n=12, 18-45y; n=15) & 63 F508 homozygous CF patients (7-11y; n=14, 12-17y; n=18, 18-45y; n=31) were included. Ht, wt, BMI, fat folds, 3-day diet composition, & fasting plasma, RBC & nasal FAs were recorded for all. In CF subjects, FEV1, DEXA & Shwachman score were obtained. Subjects were compared by disease status, gender & age group. **Data:**

AA:DHA	7-11y Control	7-11y CF	12-17y Control	12-17y CF	18-45 Control	18-45 CF
Plasma (ug/ml)	5.7+/-1.8	8.6+/-1.1**	5.2+/-1.5	5.8+/-1.5	4.1+/-1.5	6.4+/-2.5*
RBC	5.1+/-1.4	ND	4.4+/-1.3	5.1+/-1.1	3.3+/-1.2	5.1+/-1.7*
Nasal Epithelium	5.1+/-1.8	NA	4.1+/-0.9	4.8+/-0.8**	3.0+/-0.9	4.6+/-2.1*

RBC, nasal: % total FA, ug/mg protein. \*p<0.003, \*\*p<0.09. **Conclusion:** All CF FAs (means, by age) are controls. Higher CF AA:DHA is due to lower plasma, RBC and nasal DHA across age & disease severity strata with DHA trending lower as lung disease worsens. Linear regression (LR) shows no relationship between disease severity & FAs. MLR reveals plasma linolenic or RBC DHA are related to disease severity when age, gender, caloric intake & mid-arm circ (Z) are considered. Support: NIH K23, Johns Hopkins GCRC.

**Surfactant protein D (SP-D) gene mutation in pulmonary alveolar proteinosis patients and healthy controls. M.**

*Tredano*<sup>1</sup>, *M. Griese*<sup>2</sup>, *J. de Blic*<sup>1</sup>, *S. Alix*<sup>1</sup>, *J. Elion*<sup>1</sup>, *R. Couderc*<sup>1</sup>, *M. Bahuau*<sup>1</sup>. 1) Biochimie Biologie Moléculaire, Hôpital Trousseau (MT, SA, RC, MB), Pneumologie, Allergologie Pédiatriques, Hôpital des Enfants-Malades (JB), Fédération de Génétique, INSERM U458, Hôpital Robert-Debré (JE), AP-HP, Paris, France; 2) Kinderklinik und Kinderpoliklinik, Dr. von Haunerschen Kinderspital, Ludwig-Maximilians Universität, München, FRG.

Pulmonary surfactant protein D (SP-D) is a collagenous, calcium dependent, lectin (collectin), secreted by alveolar and bronchiolar cells as well as by a variety of internal or external epithelia. SP-D participates to the innate host defense and inflammatory modulation through binding micro-organisms and various organic complexes. SP-D is also present at the surface of hematopoietic cells such as macrophages, lymphocytes and neutrophils. In addition to its immune properties, SP-D participates to the structural organization of phospholipids within pulmonary surfactant. Mice rendered homozygous for an *Sftpd* targeted mutation have an abnormal surfactant clearance and exhibit a phenotype mostly similar to human pulmonary alveolar proteinosis (PAP). Based on this murine paradigm, we investigated the possible implication of *SFTPD* mutations in 19 human, familial or sporadic, juvenile or congenital, PAP. A heterozygous nonsense mutation (c.238C>T, or p.Gln80Ter) was evidenced in a sporadic case from *Blancs-des-hauts*, an endogamic subpopulation of Réunion Island with a high prevalence of PAP. In addition, and a 4-bp deletion of a consensus splice branch site (c.752-22\_-19delGACT, or IVS7-22\_-19delGACT) was identified in a sporadic case with PAP and interstitial lung disease who had also a *de novo SFTPC* mis-sense mutation (double heterozygote). Since both *SFTPD* mutations were identified as rare coding changes in the general population, although c.238C>T was more common in *Blancs-des-hauts*, we propose that these apparently deleterious changes may be innate risk factors for pulmonary disease, together with action of additional modifying genes of detrimental environmental exposures.

**Global Magnetization Transfer Imaging of the Spinal Cord in Adrenomyeloneuropathy.** *S.A. Fatemi<sup>1,2</sup>, S. Smith<sup>2</sup>, K. Zackowski<sup>3</sup>, A. Bastian<sup>3</sup>, P.C. van Zijl<sup>2</sup>, H.W. Moser<sup>1</sup>, G.V. Raymond<sup>1</sup>, X. Golay<sup>3</sup>*. 1) Neurogenetics Research Ctr; 2) F.M. Kirby Research Center; 3) Motion Analysis Lab, Kennedy Krieger Institute, Johns Hopkins University, Baltimore, MD.

Adrenomyeloneuropathy (AMN) was first recognized in 1976 as the non inflammatory adult variant of X-linked Adrenoleukodystrophy (X-ALD), a genetic disorder characterized by accumulation of very long chain fatty acids in the CNS, the adrenal cortex, and testes. The purpose of this study was to apply Global Magnetization Transfer Imaging to patients with AMN in order to visualize white matter abnormalities of the cervical spinal cord. 10 men with AMN, 10 females heterozygous for X-ALD, and 10 age and gender matched controls were evaluated neurologically and with quantitative tests of vibration threshold in the great toe and postural sway. GMT studies were conducted in all subjects on a 1.5 T MRI system using a 3D-Gradient Echo acquisition technique (TR/TR/alpha=50/12/7), with an MT pre-pulse, and 10 radiofrequency offsets. A region of interest (ROI) was selected in the dorsal column of the cervical spine from C1 C3 of the GMT images and compared to the clinical parameters. The GMT images showed signal hyperintensities in the lateral and dorsal columns of all patients demonstrating loss of MT efficiency. The GMT values of the dorsal column showed highly significant differences between the patient groups and healthy controls. Furthermore, the GMT abnormality in the dorsal column correlated with the degree of vibratory sense loss ( $r=0.67$ ,  $p=0.004$ ) and postural sway ( $r=0.53$ ,  $p=0.02$ ) assessed by quantitative sensory-motor function tests. This method enables the in-vivo visualization of white matter pathology in the spinal cord of AMN patients and may serve as sensitive tool to detect early changes in this condition.

**Genome Wide Linkage Analysis and Evidence of Gene-by-Gene Interactions in A Sample of 362 Multiplex Parkinson Disease Families.** *N. Pankratz<sup>1</sup>, W.C. Nichols<sup>2</sup>, S.K. Uniacke<sup>2</sup>, C. Halter<sup>1</sup>, J. Murrell<sup>1</sup>, A. Rudolph<sup>3</sup>, C.W. Shults<sup>4</sup>, P.M. Conneally<sup>1</sup>, T. Foroud<sup>1</sup>, Parkinson Study Group.* 1) Medical & Molec Genetics, Indiana Univ Sch Medicine, Indianapolis, IN; 2) Division of Human Genetics, Cincinnati Childrens Hospital Medical Center, Cincinnati, OH; 3) University of Rochester, Rochester, NY; 4) University of California, La Jolla, CA and the VA San Diego Healthcare System, San Diego, CA.

Parkinson disease (PD) is the second most common neurodegenerative disorder. We studied 754 affected individuals, comprising 425 sibling pairs, to identify PD susceptibility genes. Screening of the parkin gene was performed in a subset of the sample having earlier age of PD onset or a positive LOD score with a marker in the parkin gene. All subjects were evaluated using a rigorous neurological assessment. Two diagnostic models were considered for genome wide, nonparametric linkage analyses. Model I included only those individuals with a more stringent diagnosis of verified PD (216 sibling pairs) and resulted in a maximum LOD score of 3.4 on chromosome 2. Model II included all affected individuals (425 sibling pairs) and yielded a LOD score of 3.1 on the X chromosome. Our large sample was then employed to test for gene-by-gene (epistatic) interactions. A genome screen using the 23 families with PD patients having a mutation in only one allele of the parkin gene detected evidence of linkage to chromosome 10 (LOD=2.3). The 85 families with a very strong family history of PD were employed in a genome screen and, in addition to strong evidence of linkage to chromosome 2 (LOD=4.9), also produced a LOD of 2.4 on chromosome 14. A genome screen performed in the 277 families without a strong family history of PD detected linkage to chromosomes 10 (LOD=2.4) and X (LOD=3.2). These findings demonstrate consistent evidence of linkage to chromosomes 2 and X and also support the hypothesis that gene-by-gene interactions are important in PD susceptibility. This project was supported by R01 NS37167.

**The Association of COL1A1 with Otosclerosis.** *W. Chen*<sup>1</sup>, *M.J. McKenna*<sup>2</sup>, *G. Van Camp*<sup>3</sup>, *R.J.H. Smith*<sup>1</sup>. 1) Dept Otolaryngology, Univ Iowa Hosp, Iowa City, IA; 2) Department of Otology and Laryngology, Harvard Medical School, Boston, MA; 3) Department of Medical Genetics, University of Antwerp, Antwerp, Belgium.

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. Three disease loci have been identified, however causative genes and underlying mechanisms of the disease remain unknown. We have identified three single nucleotide polymorphisms (SNPs) in the regulatory regions of COL1A1 that are associated with otosclerosis based on the comparative frequency of these SNPs in 137 persons with otosclerosis and 185 controls. We tested our patient and control populations for ethnic stratification, which may lead to spurious association in case-control study, using the sum of the case-control allele frequency Cochran-Mantel-Haenszel statistics for 23 short tandem repeat polymorphism (STRP) and 2 SNP markers. These randomly chosen markers were distributed over the entire genome, but excluded the three reported otosclerosis loci, COL1A1 and COL1A2. No evidence of ethnic stratification was detected ( $p=0.5238$ ). Because the three disease-associated SNPs are found in regulatory elements, we hypothesize that they impact transcription of COL1A1. To test this hypothesis, we have introduced regulatory fragments of COL1A1 with SNP haplotypes corresponding to those found in otosclerosis patients and controls into a luciferase-based reporter (pGL3 basic). We propose to compare expression levels of luciferase from different constructs to determine whether the SNP haplotype associated with otosclerosis impacts expression of COL1A1 and subsequently bone quality. This research was supported by R01DC05218 (RJHS).

**Molecular Epidemiology of Non-Syndromic Recessive Deafness in the Indian Subcontinent: the emerging spectrum.** *M. Ghosh<sup>1</sup>, T. Smith<sup>2</sup>, R. Vijaya<sup>1</sup>, S. Riazuddin<sup>2</sup>, Z.M. Ahmad<sup>2</sup>, S. Naz<sup>2</sup>, M. Kabra<sup>1</sup>, P.S.N. Menon<sup>1</sup>, T.B. Friedman<sup>2</sup>, E.R. Wilcox<sup>2</sup>.* 1) Genetic Unit, Pediatrics, AIIMS, New Delhi, India; 2) Laboratory of Molecular Genetics, NIDCD, NIH, Rockville, Maryland, USA.

To date, more than 39 loci (DFNB1-DFNB39) and 20 genes for non-syndromic recessive hearing impairment have been identified. There are no large studies on the molecular basis of deafness in Indian families. We report here results of segregation analysis on 161 Indian families having 3 or more affected individuals from consanguineous marriages were analyzed for co-segregation with STRPs linked to known recessive deafness loci. These families were enrolled through deaf schools from various districts of the 5 Southern States- Tamilnadu, Kerala, Andhra Pradesh, Karnataka and Maharashtra, where the social custom of uncleniece marriages among Hindus and 1st cousin marriages in Muslim families occur in a significant proportion of the population. In the Southern States of India, co-segregation was commonly observed to USH1B, DFNB3 (MYO15A), DFNB4 (Pendrin) with higher prevalence in the Mysore and Hubli districts of Karnataka, and DFNB7/DFNB11 (TMC1) with 3 of the 4 families from Andhra Pradesh.

**DFNA53: A new locus for autosomal dominant hearing loss maps to 14q11.2-22.1 in a large family from China.** A. Pandya<sup>1</sup>, S.H. Blanton<sup>1</sup>, D. Yan<sup>2</sup>, X. Ke<sup>3</sup>, X.M. Ouyang<sup>2</sup>, L.L. Du<sup>2</sup>, T. Balkany<sup>2</sup>, W.E. Nance<sup>1</sup>, X.Z. Liu<sup>2</sup>. 1) Dept. Human Genetics, Virginia Commonwealth University, Richmond, VA; 2) Dept. of Otolaryngology, University of Miami, Miami, FL; 3) Dept. of Otolaryngology, Beijing Medical School, Beijing, China.

Hearing loss (HL) has many known genetic and environmental causes and affects at least 30% of the population at some time in their lives. It is a genetically heterogeneous disorder, with perhaps 1% (i.e. at least 300) of the total genes suspected of causing deafness. Half of all profound deafness is genetic, and 15-20% of non-syndromic HL is transmitted as an autosomal dominant trait. At present 37 loci for dominant HL have been mapped, and 18 genes have been identified. We report on yet another novel dominant locus at 14q11.2-22.1 for HL in a large multi-generation Chinese family ascertained from Northern China. All affected individuals had onset of HL between the ages of 18-22 years, which was moderate to profound in severity and progression in severity was noted. Intra-familial variation occurred in the age of onset and severity of HL. There was no vestibular dysfunction or other associated abnormalities. Linkage analysis was performed with Simwalk2 assuming a penetrance of 0.9 without phenocopies. A multipoint maximum LOD score of 5.35 was obtained at marker D14S1280 after a genome wide scan. The locus maps to an approximately 50cM region, flanked by the markers D14S742 and D14S587. As this region overlaps with the DFNA9 locus, the coding region of COCH gene was sequenced and excluded in affected probands, with no mutations identified. The interval for DFNA53 does not overlap with the region for DFNA23 which maps telomeric to our locus. We are currently fine mapping with additional markers to narrow the critical region and are screening for mutations in plausible candidate genes.

**Monogenic cochlear otosclerosis: clinical and linkage analysis in a large Greek family.** *M.B. Petersen<sup>1</sup>, K. Van Den Bogaert<sup>2</sup>, V. Iliadou<sup>3</sup>, N. Eleftheriades<sup>3</sup>, G. Aperis<sup>1</sup>, K. Vanderstraeten<sup>2</sup>, E. Franssen<sup>2</sup>, M. Grigoriadou<sup>1</sup>, A. Pampanos<sup>1</sup>, J. Economides<sup>4</sup>, T. Iliades<sup>3</sup>, G. Van Camp<sup>2</sup>.* 1) Dept Genetics, Inst Child Health, Athens, Greece; 2) Dept Med Genetics, Univ Antwerp, Belgium; 3) Dept Otorhinolaryngol, Aristotle Univ Thessaloniki, Greece; 4) Dept Audiol-Neurootol, "Aghia Sophia" Children's Hosp, Athens, Greece.

Otosclerosis, which is a common cause of hearing impairment among white adults, can be considered as a multifactorial disease. About 50% of patients have a positive family history, but until now only three autosomal dominant otosclerosis loci have been reported: OTSC1 (15q25-q26), OTSC2 (7q34-q36), and OTSC3 (6p21-p22). We describe a large multigenerational Greek family with autosomal dominant cochlear otosclerosis. After informed consent, 46 family members were examined. The hearing loss in this family appears in childhood at the age of 10 years as a conductive hearing loss, but soon becomes mixed. Because the additional sensorineural component is progressive, this finally leads to a pure sensorineural hearing loss in some family members. Audiometrical analysis of the affected individuals, by Multiple Linear Regression (MLR) analysis and construction of Age-Related Typical Audiograms (ARTA), showed an age-independent conductive component and a progressive frequency-specific sensorineural component. In addition to this detailed clinical analysis, a genetic linkage analysis was performed for the three known otosclerosis loci as well as for the COL1A1 and COL1A2 genes. These latter genes, involved in the pathogenesis of osteogenesis imperfecta, were analyzed to study a previously suggested shared etiology between otosclerosis and osteogenesis imperfecta. Three microsatellite DNA polymorphisms for each OTSC locus or collagen gene were analyzed, and two-point lod scores excluded linkage to each of these known loci. From a clinical point of view, this is the first detailed audiometrical analysis in a monogenic otosclerosis family. From a genetic point of view, exclusion of the three known otosclerosis loci shows that otosclerosis is a genetically heterogeneous disease involving at least four different genes.

**Comparison between various strategies for the disease-gene mapping using linkage disequilibrium analyses: studies on adenine phosphoribosyltransferase deficiency used as an example.** *S. Kuno<sup>1</sup>, A. Taniguchi<sup>2</sup>, A. Saito<sup>3</sup>, S. Tsuchida-Otsuka<sup>2</sup>, N. Kamatani<sup>4</sup>.* 1) Laboratory for Clinical Genome Informatics, Translational Research Informatics Center, Foundation for Biomedical Research and Innovation, Kobe, Japan; 2) Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan; 3) Advanced Research Laboratory, Hitachi Ltd., Tokyo, Japan; 4) Division of Genomic Medicine, Department of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan.

Recently, linkage disequilibrium analyses have been used to detect disease-causing loci based on common disease common variant hypothesis. SNPs, STRPs and VNTR have been mainly utilized as markers. The three types of markers have different characteristics. SNPs are present at the highest frequency while STRP and VNTR have multiple alleles at a single locus. Furthermore, the mutation rates at SNP loci are likely to be very low while those for STRP loci may be rather high. What kind of markers and what methods should be used for the effective analyses? To answer this question, we have to apply the methods to the practical data obtained from human population. We extensively performed linkage disequilibrium and haplotype analyses on adenine phosphoribosyltransferase (APRT) genes in both control and the deficiency subjects. To examine the power to detect disease-causing loci, we analyzed SNPs, STRPs and VNTR within and around the APRT gene. When only SNPs were used, P values did not necessarily show significant difference at loci close to the mutation site for APRT\*J that is exclusively observed among Japanese. However, the examination of the same samples with haplotypes based on the haplotype block data gave sufficient significance. In the case of STRP and VNTR, some single marker loci showed significant difference. However, the combination of the SNP, STRP and VNTR for the construction of haplotypes gave more evident results. Our study suggested that the use of haplotype analysis based on the haplotype-block structure is more powerful than single marker locus analysis for the detection of disease-related loci.

**Cartographer: A tool to generate marker maps based on the physical, genomic location of markers.** *A. Metzidis, S. Sammalisto, M. Perola, L. Peltonen, J. Saharinen.* Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland.

A properly ordered marker map is essential for successful linkage analyses. Multipoint linkage analyses are particularly sensitive to the marker order. Typically markers are ordered by using genetic maps or by building maps by hand using online genome databases. Existing genetic marker maps have been shown to be inconsistent with the actual genome sequence, and manual construction of maps is time consuming and error prone. To solve this problem we have developed Cartographer, a web-service that automatically builds a physically-ordered marker map, generated from a list of marker names, using the localization of full-marker and marker-primer sequence data. Cartographer is a web interface to a database derived from data obtained from the UCSC Genome Annotations Database. The UCSC database has annotations for nearly 200,000 STS markers. Location information exists for nearly half of these. Cartographer accepts a simple marker-name list as input, and returns the markers physically ordered with supplemental information including Decode and Marshfield Map positions, chromosomal location and DBSTS ID, among others. In addition, missing genetic map positions are interpolated based on those of neighboring markers, taking into account the physical distance between them. Cartographer works by first mapping marker aliases to their canonical names. Recorded BLAT localizations of marker sequence data are then used to discover the physical location for each marker. Once ordered, marker annotations are retrieved from the database, coupled with the location information, and presented to the user via a web page. The results may then be downloaded as ASCII text files. Cartographer has been developed for GNU/Linux operating systems using PHP, PostgreSQL and GNU tools. Preliminary tests of Cartographer show a high success rate typically over 99% of markers get accurately located and properly ordered. Accurate maps are built in seconds. Currently we are expanding the database to increase the tool's accuracy. SNP markers will be included in the near future. See [www.bioinfo.helsinki.fi/cartographer](http://www.bioinfo.helsinki.fi/cartographer).

**Database Schema for Management and Storage of Single Nucleotide Polymorphisms.** *T. Niu*<sup>1,2,3</sup>, *K. Baclawski*<sup>2</sup>, *Y. Feng*<sup>3</sup>, *H. Wang*<sup>3</sup>. 1) Div. of Prev. Med., Dept. of Med., Brigham & Women's Hosp., Harvard Med. Schl., Boston, MA; 2) Coll. Computer Sci., Cullinane Hall, Northeastern Univ., Boston, MA; 3) Program for Population Genetics, Harvard Schl. of Public Hlth., Boston, MA.

Single nucleotide polymorphisms (SNPs) are the most frequent DNA sequence variations in the human genome. SNPs have a wide range of biomedical applications. The sequencing of the human genome has resulted in huge volumes of bioinformatics data, in general, and SNP data in particular. In spite of the importance of SNPs, there has been limited progress in developing effective techniques for managing the enormous amount of SNP data now being generated. Many labs have devised their own solutions for storing and distributing SNP data, but there is a lack of generic software solutions. In this paper, we describe the design of a database system for SNP information, called the SNP database (SNPDB). The purpose of the SNPDB is to support the acquisition, storage and retrieval of large amounts of SNP information. Because of the complexity of SNP data, an ontology for SNP information was developed in the Web Ontology Language (OWL). Ontologies have significant advantages for modeling complex data, for the interchange of this data between different systems and for integrating complex data from different sources. However, because modern database management systems are based on the relational model, the SNP ontology was mapped to the relational model. SNP data is used for many purposes and is often integrated with other biological data. The paper concludes with some examples of SNP queries to illustrate how the SNPDB is used for these purposes.

**An integrated tool to generate and present LD maps.** *L. Warren<sup>1</sup>, A. Wooster<sup>2</sup>, D. Zaykin<sup>1</sup>, P. St. Jean<sup>1</sup>.* 1) Population Genetics, GlaxoSmithKline, RTP, NC; 2) Bioinformatics, GlaxoSmithKline, RTP, NC.

Case control based pharmacogenetics studies and disease gene association mapping rely on the linkage disequilibrium between some SNP markers and the drug response or disease causing genes. With more than 1.8 million publicly available SNPs currently, there is a need to have an efficient tool to generate and present LD patterns for large numbers of SNPs. There are two challenges: first, to develop an efficient algorithm to calculate LD for hundreds of thousands of SNPs; and second, to construct a user-interface that allows analysts to investigate millions of LD pairs.

The linkage disequilibrium coefficient for alleles A and B is defined as  $D_{AB} = P_{AB} P_A P_B$ , where  $P_{AB}$  is the gametic frequency and  $P_A P_B$  is the product of the allele frequencies. Because its absolute value depends on the allele frequencies,  $D_{AB}$  is not suitable for comparing levels of LD across marker pairs. Alternatively, two standardized parameters,  $r^2$  and  $D'$  are often used to measure the strength of LD.  $r^2$  is the standard Pearson's correlation coefficient and its calculation is straightforward.  $D'$  can be obtained by the EM algorithm, which is also the rate limiting step when calculating  $D'$  for millions of SNP pairs. In addition,  $D'$  from EM relies on the assumption that each individual SNP is in HWE, which may or may not be valid in reality. Here, we propose a method that can calculate the composite  $D'$  on the fly for a large number of SNPs. An additional advantage of using composite  $D'$  is that it does not rely on the HWE assumption. To assess LD significance, P-values are also calculated for the estimated parameters.

To present the LD patterns for a large number of SNPs in a dynamic and user-interactive fashion, we have integrated the Spotfire™ data visualization tool. Several ways to present LD and average LD across regions will be illustrated. These plots can also be directly imported into other applications such as Powerpoint, Word, etc.

**Familial Venous-occlusive Disease of the Liver with Immunodeficiency: Localization to a 1Mb Region of 2q36.3-37.1 via Homozygosity Mapping.** *T. Roscioli<sup>1</sup>, M.F. Buckley<sup>1</sup>, P.J. Taylor<sup>1</sup>, E. Kirk<sup>2</sup>, J. Ziegler<sup>3</sup>, M. Wong<sup>4</sup>, J.A. Donald<sup>5</sup>, R. Lindeman<sup>1</sup>.* 1) Centre for Thrombosis and Vascular Biology, University of NSW, Sydney, Australia; 2) Department of Medical Genetics, Sydney Children's Hospital Randwick, NSW, Australia; 3) Department of Immunology and Infectious Disease, Sydney Children's Hospital Randwick, NSW, Australia; 4) Department of Immunology and Infectious Disease, The Children's Hospital Westmead, NSW, Australia; 5) Department of Biological Sciences, Macquarie University, NSW, Australia.

We present three families of Middle Eastern origin with an autosomal recessive form of venous-occlusive disease of the liver with immunodeficiency (VODI) requiring treatment with intravenous immunoglobulin. Multiple consanguinity in two families, and a shared geographic origin and ethnic origin of all suggests the presence of a founder mutation. This cohort of consanguineous families with a common ethnic origin has allowed the identification of a single 1 megabase candidate interval on human chromosome 2q36.3-37.1 by homozygosity mapping. Sequencing of the eight candidate genes in the critical region is in progress. 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. This histological appearance was first described in VODL secondary to ingestion of beverages containing pyrrolizidine alkaloids. VODL is now most often seen within twenty days of a post bone marrow transplant and it is an important cause of morbidity and mortality after this therapy. The histological features of VODI are indistinguishable from those of VODL after BMT. This suggests that VODI may provide important information regarding the pathology of the VODL and that defining the genetic aetiology of VODI could lead to the identification of individuals most at risk of hepatic venous-occlusive disease in the context of bone marrow transplantation.

**Fetal akinesia with anterior horn cell loss and congenital malformation in male siblings: Severe X-linked spinal muscular atrophy (XL-SMA) or a new disease entity?** *J. Gerritsen*<sup>1</sup>, *E. Estrella*<sup>2</sup>, *M.E. Ahearn*<sup>2</sup>, *K.O. Yariž*<sup>2</sup>, *L. Baumbach*<sup>2</sup>. 1) Univ. of Calgary, Calgary, Alberta, Canada; 2) Univ. Miami Sch. Med., Miami, FL.

We report clinical and molecular investigations of two brothers (born to unrelated parents) with prenatal-onset anterior horn cell loss. **Clinical Summary:** Prenatal diagnosis of arthrogyriposis was suspected. Both boys shared physical features associated with fetal akinesia: multiple contractures, distinctive craniofacial appearance, gracile ribs on X-ray, and undescended testes/inguinal hernia. Brother 1 had a duplicated left thumb and horseshoe kidney. Both boys displayed unresolving hypotonia at birth. Gag reflexes were also absent, and the ability to protect the upper airway was affected. Respiratory insufficiency resulted in ventilator dependence. Brother 1 also had significant apneas and bradycardias. He died at 2 weeks of age. Brother 2 is currently still living at 5 months old, but awaiting home ventilation. EMG/NCV studies in both were consistent with anterior horn cell loss. Results of muscle biopsy from both boys showed perimyseal fibrosis, atrophied fibers, and nonspecific variation in fiber diameter. Autopsy of brother 1 noted profound loss of anterior horn cells, degeneration of the CN12 nucleus, and a mildly atrophic brain with poor white-gray matter differentiation. **Laboratory Studies:** Both boys had normal karyotypes. Brother 1 had normal CK values, normal myotonic dystrophy DNA testing, and a metabolic evaluation that was normal. Loss of anterior horn cells prompted study of SMA syndromes. Neither boy had deletion of *SMN* exons 7 and 8. Linkage to the XL-SMA region in Xp11.3-Xq11.2 was then performed. Six dinucleotide repeat markers, previously shown to link to this region, were evaluated using the ABI 3100 and GeneMapper software. Haplotype analysis revealed discordant inheritance of these markers in the brothers. Additional DNA markers in the region are being studied to rule out a small recombination event which might link this family to the XL-SMA critical region, as well as other X chromosome markers, to determine if this family represents a new X-linked disease phenotype.

**Genetic linkage of kyphoscoliosis.** *C.M. Justice<sup>1</sup>, B. Marosy<sup>2</sup>, S. Novak<sup>2</sup>, P. Boyce<sup>3</sup>, J. Pettengill<sup>3</sup>, K.F. Doheny<sup>3</sup>, E.W. Pugh<sup>3</sup>, A.F. Wilson<sup>1</sup>, N.H. Miller<sup>2</sup>.* 1) Genometrics Section, NHGRI/NIH, Baltimore, MD; 2) Department of Orthopaedic Surgery, Johns Hopkins University, Baltimore, MD; 3) CIDR, IGM, Johns Hopkins University SOM, Baltimore, MD.

Thoracic kyphoscoliosis is an unusual deformity in which a lateral curvature of the spine is seen in conjunction with thoracic hyperkyphosis in otherwise normal individuals. As part of a large collaborative study of familial idiopathic scoliosis, 202 families (1198 individuals) with at least two affected individuals with a lateral curvature 10 degrees were ascertained and clinically characterized. Ten of these families (68 individuals) had at least one individual with kyphoscoliosis (lateral curvature 10 and thoracic kyphosis 40 ) documented prior to skeletal maturity. Within this group, the average degree of lateral curvature was 48 for individuals with kyphoscoliosis, and 31 for individuals without kyphoscoliosis. The average degree of kyphosis was 53. A genome-wide screen was performed at the Center for Inherited Disease Research using a modified CHLC v.9 marker set consisting of 391 short tandem repeat markers. Sib-pair analysis of the genomic screen of the initial group suggested linkage to regions on chromosomes 2, 5, 13, and 17. In an effort to further restrict the phenotype, the subset was limited to families in which two or more individuals had kyphoscoliosis (7 families; 53 individuals). This resulted in an increase in significance of the p-values for all the regions suggestive of linkage. An additional 25 markers were genotyped in the regions of interest, which helped narrow the candidate regions. The most significant p-values were obtained for regions on chromosomes 5 and 13. In an attempt to narrow further the candidate region and evaluate associations with kyphoscoliosis, 145 SNPs were genotyped in an ~10 Mb region on chromosome 5p13.

**Mapping of a new locus for autosomal recessive nonsyndromic mental retardation in the chromosomal region 19p13.12-p13.2: further genetic heterogeneity.** *L. Basel-Vanagaite*<sup>1, 2</sup>, *A. Alkelai*<sup>3</sup>, *R. Straussberg*<sup>2,4</sup>, *N. Magal*<sup>3,4</sup>, *D. Inbar*<sup>5</sup>, *M. Mahagna*<sup>5</sup>, *M. Shohat*<sup>1,4</sup>. 1) Dept Medical Genetics, Rabin Medical Ctr, Petah Tikva, Israel; 2) Neurogenetics Clinic, Schneider Childrens Medical Center of Israel, Petah Tikva, Israel; 3) Felsenstein Medical Research Center, Petah Tikva, Israel; 4) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; 5) Neuropediatrics Unit, Schneider Childrens Medical Center of Israel, Petah Tikva, Israel.

We have identified and evaluated clinically four consanguineous families of Israeli Arab origin with autosomal recessive nonsyndromic mental retardation (NSMR) that included a total of 10 affected and 22 unaffected individuals. All the families originate from the same small village and bear the same family name. Association of the condition in these families with the two known autosomal recessive NSMR loci on chromosomes 3p25-pter and 4q24 (neurotrypsin gene) was excluded. Linkage of the disease gene to chromosome 19p13.12-p13.2 ( $Z_{\max} = 7.06$  at  $\theta = 0.00$  for the marker D19S840) was established. All the affected individuals were found to be homozygous for a common haplotype for the markers cen-RFX1-D19S840-D19S558-D19S221-tel, suggesting that the disease is caused by a single mutation derived from a single ancestral founder in all the families. Recombination events and a common disease-bearing haplotype defined a critical region of 2.6 Mb between the loci D19S226 proximally and D19S1165 distally. Based on the data reported here and in other studies, we conclude that there are at least three genetic loci that can cause autosomal recessive NSMR. Additional autosomal loci are expected to be involved in the etiology of NSMR. Because of the increasing number of possible autosomal recessive NSMR loci, we suggest that formal classification of these genes should be introduced. We suggest the nomenclature ARNSMR1 for the NSMR associated with the locus on chromosome 3p25-pter, ARNSMR2 for that associated with the neurotrypsin gene, and ARNSMR3 for that associated with the locus on chromosome 19p13.12-p13.2.

**Analysis of mapped SCA loci in an Italian family with autosomal dominant Spinocerebellar ataxia.** *C. Cagnoli*<sup>1</sup>, *C. Geller*<sup>2</sup>, *C. Mariotti*<sup>2</sup>, *A. Castucci*<sup>2</sup>, *C. Michielotto*<sup>1</sup>, *S. Di Donato*<sup>2</sup>, *F. Taroni*<sup>2</sup>, *N. Migone*<sup>1</sup>, *A. Brusco*<sup>1</sup>. 1) Dept. Genetics Biol. & Bioch., University of Torino, & Az. Osp. San G. Battista, Torino, Italy; 2) Dept. Biochemistry & Genetics, Istituto Nazionale Neurologico C. Besta, Milano, Italy.

The spinocerebellar ataxias (SCAs) are a group of neurodegenerative disorders, characterized by progressive cerebellar ataxia variably associated with other neurological features. SCAs are genetically heterogeneous comprising 19 distinct subtypes (SCA1-8 and SCA10-14, 16, 17, 19, 21, 22, and SCA-FGF14). Eleven of them have been assigned to specific genes (*SCA1-3, 6-8, 10, 12, 14, 17, and FGF14* genes). We have studied a three-generation Italian family with 6 SCA members and 5 non-affected relatives. Clinical evaluation of the patients showed slowly progressive cerebellar ataxia of gait and limbs with onset between 20 and 25 yr, ocular movement abnormalities, such as nystagmus and ophtalmoplegia, and hyperreflexia. Brain MRI demonstrated cerebellar atrophy. Genetic testing excluded expansions in the known SCA1-3, 6-8, 10, 12, and 17 genes. Anticipation was not a prominent feature, but large CAG/CTG expansions (40 rep.) in unknown genes were ruled out by Rapid Expansion Detection (RED) analysis. In addition, we performed linkage analysis to 8 of the known loci SCA4, SCA5, SCA11, SCA13, SCA14, SCA16, SCA19, SCA21, and FGF14, using microsatellite markers spanning the candidate regions. Haplotype analysis and two-point LOD score -2.0 ruled out a linkage with SCA4, SCA5, SCA14, SCA16, SCA21, and FGF14. The SCA11, SCA13, and SCA19 loci could not be completely excluded, due to the presence of non-informative markers. However, including the 5 available healthy members (age 42-50), and 90% penetrance, linkage could be excluded also for SCA11 and SCA13 loci. The SCA19 locus is still under investigation to cover its 35 cM extension. Additional affected relatives will be collected to complete this study, which may lead to a new putative SCA locus.

**Genetic Linkage of Fibromyalgia to the Serotonin Receptor 2A Region on Chromosome 13 and the HLA Region on Chromosome 6.** *D.M. Dudek<sup>1</sup>, L.M. Arnold<sup>2</sup>, S.K. Iyengar<sup>1</sup>, M.A. Khan<sup>1</sup>, I.J. Russell<sup>3</sup>, M.B. Yunus<sup>4</sup>, J.M. Olson<sup>1</sup>.* 1) Dept. of Epidemiology and Biostatistics and Dept. of Medicine, Case Western Reserve University, Cleveland, OH; 2) Dept. of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, OH; 3) Dept. of Medicine, University of Texas Health Sciences Center, San Antonio, TX; 4) Dept. of Medicine, University of Illinois College of Medicine, Peoria, IL.

The serotonin neurotransmitter and autoimmune systems have been suggested as important to the etiology of fibromyalgia (FM). To assess the genetic basis of FM, we examined markers spanning genomic regions for the serotonin receptor 2A (HTR2A), the serotonin transporter (HTTLPR), and the HLA. 80 multicase families confirmed by ACR criteria were collected as part of the Fibromyalgia Family Study. Severity and quality of pain were measured using a visual analog scale, the McGill short form, and an anatomical sketch (AS). Other measures included the SF-36, the Multidimensional Assessment of Fatigue, the Beck Anxiety (BAI) and Depression (BDI) Inventories, and the Jenkins sleep scale. Participants were also evaluated using standard criteria for the presence of chronic fatigue syndrome (CFS), irritable bowel syndrome (IBS), and restless legs syndrome. Linkage analyses were conducted using LODPAL (S.A.G.E. 2002). Exploratory data analyses determined that a subset of largely-unrelated traits (age-of-onset, AS, BDI, and IBS) exhibited the best evidence of familial aggregation among FM patients and were included as covariates in the affected-relative-pair linkage analysis. No evidence for linkage was found in the HTTLPR region. In the HLA region, results suggest that families of later age-of-onset are linked (lod=3.02, p=.00057). In the HTR2A region, results suggest that linked families are of earlier age-of-onset (lod=1.96, p=.0068), report less pain (lod=3.33, p=.0028) and lower BDI (lod=1.86, p=.0086), and lack IBS (lod=2.46, p=.0021); the best, most parsimonious linkage model includes age-of-onset and AS (lod=5.56, p=.000057). These results support the hypothesis that HTR2A predisposes to an earlier-onset form of FM with milder reported symptoms.

**X chromosome scanning in a family with Lujan-Fryns syndrome recurrence suggests two possible loci in Xp22.3 and Xq28.** A. Ferlini<sup>1</sup>, A. Ravani<sup>1</sup>, A. Venturoli<sup>1</sup>, M. Neri<sup>1</sup>, P. Rimessi<sup>1</sup>, F. Gualandi<sup>1</sup>, D. DeGrandis<sup>2</sup>, E. Calzolari<sup>1</sup>. 1) Section of Medical Genetics, University of Ferrara, Ferrara, (Italy); 2) Dipartimento di Neuroscienze, Ospedale Civile, Rovigo (Italy).

Lujan-Fryns syndrome represents a mendelian condition characterized by aspecific mental retardation, marfanoid habitus, hypotonia, craniofacial anomalies and others inconsistent features. The disease recognizes a transmission compatible with an X-linked inheritance (OMIM\*309520). Although candidate chromosomal regions/genes have been proposed as being associated with this disease (FRAXE and chromosome 5p deletions) the genetic aetiology of the syndrome is still unknown. We performed a wide genome scanning analysis of the X chromosome in a large pedigree with Lujan-Fryns syndrome recurrence compatible with an X-linked mode of inheritance (two affected males, uncle and nephew). Haplotype sharing in nine family members, based on VNTRs segregation analysis (four panels, Applied Biosystem), allowed us to exclude almost all the X chromosome apart from the Xp22.3 and Xq28 regions. The data we provide reduced the X chromosome interval containing the Lujan-Fryns gene. In keeping with observations already reported, the Xq28 possible mapping might confirm the involvement of the FRAXE site. This approach will enable us to refine the actual mapping in order to search for candidate genes.

**Analysis of the interleukin-1 gene complex in LOAD Brazilian patients.** *A.L. Nishimura<sup>1</sup>, F.B. Mury<sup>1</sup>, M. Mitne-Neto<sup>1</sup>, J.R.M. Oliveira<sup>2</sup>, R. Nitrini<sup>3</sup>, V.S. Bahia<sup>3</sup>, P.R. Brito-Marques<sup>4</sup>, M. Neri<sup>4</sup>, S.M.M. Fertuzinhos<sup>1</sup>, M. Zatz<sup>1</sup>.* 1) 1- Human Genome Research Center, Institute of Biosciences, University of S.Paulo, Brazil; 2) 2- Neurology Department of the David Geffen Medical School, UCLA, USA; 3) 3- Department of Neurology, Faculty of Medicine, University of S.Paulo, Brazil; 4) 4- Center of cognitive neurology and of behavior, University of Pernambuco, Brazil.

Several studies have attempted to find a relationship between inflammatory pathways and psychiatric disorders. Recently, three polymorphisms of the interleukin-1 (IL-1) gene complex, including the IL-1 alpha, IL-1 beta and IL-1 receptor antagonist (IL-1RA) have been associated to different psychiatric disorders. The IL-1RA has a variable number of tandem repeats in intron 2, the IL-1 alpha gene has a base exchange at the position -889 and IL-1 beta gene has a base exchange at the position -511. These polymorphisms have been associated to late onset Alzheimer disease (LOAD) pathogenesis inducing the translation and processing of the beta-amyloid precursor protein with possible implications on the progression of the plaque and tangle formation. Moreover, the cytokines can modify the metabolism of neurotransmitters or influence neural development. Here we analyzed 120 LOAD patients (mean age 68.78) diagnosed according to NINCDS-ADRDA criteria. Neurological and neuropsychological testing including Mini-Mental State Exam (MMSE) and Clinical Dementia Rating (CDR) were performed in order to classify their cognitive impairment. The 120 healthy controls (mean age 72.39.75) were selected based on the MMSE and/or Blessed Scale and were matched with the patients group for age, sex, socio-cultural level and ethnic background. All patients and controls were originated from two Brazilian States, São Paulo and Pernambuco. The preliminary statistical analysis did not show any statistically significant association between the polymorphisms of the interleukin-1 gene complex and LOAD Brazilian patients ( $P=0.183$ ,  $OR=1.303$ , 95% C.I.=0.896-1.895 for IL-1 alpha;  $P=0.210$ ,  $OR=0.759$ , 95% C.I.=0.502-1.148 for IL-1 beta and  $P=0.3$  for IL-1RA). Supported by FAPESP-CEPID, CNPq, PRONEX.

**Clinical and genetic study of an Italian family with a new form of autosomal dominant complicated Spastic Paraplegia.** A. Magariello<sup>1</sup>, L. Passamonti<sup>2</sup>, R. Mazzei<sup>1</sup>, A. Patitucci<sup>1</sup>, FL. Conforti<sup>1</sup>, M. Bellesi<sup>2</sup>, AL. Gabriele<sup>1</sup>, T. Sprovieri<sup>1</sup>, G. Peluso<sup>1</sup>, M. Caracciolo<sup>1</sup>, E. Medici<sup>2</sup>, F. Logullo<sup>2</sup>, G. Di Palma<sup>1</sup>, L. Provinciali<sup>2</sup>, M. Muglia<sup>1</sup>. 1) ISN, National Research Council, Mangone, Cosenza, Italy; 2) Department of Neurological Sciences, A.O. Umberto I, Ancona, 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. Clinically, HSP is classified as pure form if neurological impairment is limited to progressive lower extremity and complicated if additional symptoms such as seizures, dementia, amyotrophy, extrapyramidal disturbance or peripheral neuropathy also occur. HSP is inherited with autosomal dominant, autosomal recessive or X-linked traits. To date, 10 loci associated with autosomal dominant HSP (ADHSP) have been demonstrated, two of them (SPG9 and SPG17) have been associated with complicated HSP form. The first type was associated with cataracts, gastroesophageal reflux and motor neuropathy and linked to chromosome 10q23, whereas the second form was accompanied by amyotrophy of hand and feet muscles (Silver syndrome) and linked to chromosome 11q12. We studied an Italian pedigree with ADHSP to determine if this variant of HSP was linked to any of the known autosomal dominant loci. The HSP phenotype in this family was complicated by axonal motor neuropathy without gastroesophageal reflux and cataracts, and by distal muscle weakness and wasting in the lower limbs. Genetic linkage analysis of this family was carried out with DNA markers from current-known ADHSP loci. Negative lod scores were obtained with all microsatellite markers tested. Our family was not linked to any of the known loci associated with autosomal dominant HSP. These data demonstrate further locus heterogeneity for ADHSP and suggest that the current phenotype is caused by another, as yet unmapped, gene.

Program Nr: 1751 from 2003 ASHG Annual Meeting

**No genetic association found between five SNPs on COMT gene and schizophrenia in Korean population.** *E.J. Joo*<sup>1</sup>, *S.H. Jeong*<sup>2</sup>, *Y.M. Ahn*<sup>3</sup>, *Y.S. Kim*<sup>3</sup>. 1) Psychiatry, Eulji Medical School, Seoul, South Korea, MD; 2) Chook-Ryung Evangelical Hospital, Namyangju, South Korea, MD; 3) Psychiatry, Seoul National University, Seoul, South Korea, MD.

**Objective:** This study aimed to explore genetic relation between schizophrenia and COMT(Catechol-O-methyl transferase) gene which plays an important role in metabolizing dopamine, one of the most intriguing neuro-transmitters for schizophrenia. **Methods:** Genotyping was done for five SNPs on COMT gene for 254 patients with schizophrenia and 252 normal controls by SNaPShot method. Patients with schizophrenia were collected when they agreed to participate in this study and wrote the informed consent. DSM-IV diagnosis of schizophrenia was made based on clinical information and/or DIGS(Diagnostic Interview for Genetic Studies). Normal controls were collected among voluntary participants in this study. Subjects who have any kinds neurologic disorder or medical illness possibly having psychiatric features or head trauma were excluded from this study. Allele frequencies, genotype frequencies and simulated haplotype frequencies were compared between patients with schizophrenia and normal controls. **Results:** No significant difference between patients with schizophrenia and normal controls in terms of allele frequencies, genotype frequencies and haplotype frequencies was found in our sample. **Conclusion:** No genetic association between five SNPs on COMT gene and DSM-IV diagnosis of schizophrenia among Koreans was found in this study.

**A newly described neurologic disease Autosomal Dominant Acute Necrotizing Encephalopathy (ADANE) maps to 2q12.1-2q13.** *D.E. Neilson<sup>1,2</sup>, H.S. Feiler<sup>3</sup>, K.C. Wilhelmsen<sup>3,4</sup>, D.S. Kerr<sup>1</sup>, M.L. Warman<sup>1,2</sup>.* 1) Depts. of Pediatrics; 2) and Genetics, Case Western Reserve University School of Medicine, University Hospitals of Cleveland, Cleveland, Ohio; 3) Ernest Gallo Clinic and Research Center; 4) Dept. of Neurology, University of California San Francisco School of Medicine.

We identified a family segregating the novel, incompletely penetrant, neurologic disease ADANE. (Neilson et al. *Neurology*. 2003 Jul;61(2):226-230). ADANE is clinically similar to the sporadically occurring disorder Acute Necrotizing Encephalopathy (ANE). In both disorders, apparently healthy children become comatose following a febrile illness and have necrotizing lesions detectable by MRI, or at autopsy, in their thalami and brainstems. Outcomes of ADANE and ANE include full recovery, permanent brain damage, or death. Infectious triggers, e.g. influenza A, are associated with ANE and have been seen in some family members with ADANE. A muscle biopsy of one child with ADANE demonstrated loose coupling of oxidative phosphorylation. Leigh syndrome (subacute necrotizing encephalopathy) is clinically distinct from ADANE. We now report the map location for ADANE. We used DNA from affected individuals and obligate carriers (based on position within the pedigree) to perform a whole genome scan at 5 cM resolution. Linkage was detected to two markers D2S2264 and D2S293. Saturating this region with markers at 1 Mb intervals yielded a lod score of 3.6 and refined the candidate interval between D2S135 and D2S1896. Of 33 RefSeq genes contained within this interval, none previously have been associated with heritable neurodegenerative disease. We evaluated two genes (BCL2L11 and ST6GalII) having possible roles in apoptosis and viral recognition by sequencing their exons and flanking introns. No disease causing mutations were identified; however, we found an intronic polymorphism within BCL2L11 that is recombinant in one individual enabling us to further narrow the candidate interval. The ADANE locus is in a 6 Mb interval between D2S135 and BCL2L11. The identification of the responsible gene should facilitate our understanding of ADANE's pathogenesis and may provide insight about the more common disorder ANE.

**The analysis of polymorphisms of synuclein family genes in multiple system atrophy (MSA).** *H. Ohtake*<sup>1</sup>, *O. Onodera*<sup>1,2</sup>, *A. Kakita*<sup>2,3</sup>, *A. Hasegawa*<sup>1</sup>, *S. Igarashi*<sup>1,2</sup>, *T. Ozawa*<sup>1</sup>, *K. Okuizumi*<sup>1</sup>, *H. Takahashi*<sup>3</sup>, *S. Tsuji*<sup>4</sup>, *M. Nishizawa*<sup>1</sup>. 1) Neurology; 2) Molecul Neurosci; 3) Pathology, Brain Res Inst, Niigata Univ, Niigata; 4) Neurology, Univ Tokyo, Tokyo, Japan.

MSA is an adult onset neurodegenerative disease, featuring parkinsonism, ataxia, and autonomic failure, in any combination. The condition is progressive and responds poorly to treatment. Argyrophilic inclusion bodies in oligodendroglia, glial cytoplasmic inclusions (GCI), have been established as the pathognomonic pathological findings in MSA. GCI are immunoreactive for ubiquitin and -synuclein (SNCA). The presence of SNCA in GCI suggests a link with Parkinson's disease (PD), and dementia with Lewy bodies (DLB), in which SNCA is also the major component of Lewy bodies. This has led to the term synucleinopathy to embrace this group of conditions. In the rare autosomal dominant PD families, two missense mutations of SNCA have been reported. Others and we have reported the missense mutations of -synuclein (SNCB) and -synuclein (SNCG) in a few sporadic DLB patients. Very recently a missense mutation in synphilin-1 (SNCAIP) gene has been reported in two sporadic PD patients. In addition, the association between SNCA haplotype and development of sporadic PD has been reported. With this background, we hypothesized that MSA, another type of sporadic synucleinopathy with GCI, might also associate with genetic variation of synuclein family genes. We have collected 48 Japanese patients with MSA, including 11 pathologically proven cases, which met Gilman's criteria of MSA. We analyzed the entire coding region of SNCA and 27 single nucleotide polymorphisms (SNPs) surrounding the synuclein family genes, SNCA, SNCB, SNCG and SNCAIP in these cases. We performed nucleotide sequence analysis of SNCA gene using total RNA or genomic DNA. Genotyping was performed by the Taqman Allelic Discrimination Method. We did not identify any mutations in the entire coding region of SNCA in 11 pathologically confirmed MSA cases. We did not find any significant evidence for linkage disequilibrium associated with the SNPs in the synuclein family genes compared with 48 healthy Japanese controls.

**Genome-wide microsatellite association studies for Parkinson's disease by using the pooled DNA method. Y.**

*Hirota<sup>1</sup>, Y. Momose<sup>2</sup>, W. Satake<sup>1</sup>, M. Murata<sup>3</sup>, G. Tamiya<sup>4</sup>, T. Ikuta<sup>4</sup>, S. Makino<sup>4</sup>, I. Mizuta<sup>1</sup>, M. Yamamoto<sup>5</sup>, N. Hattori<sup>6</sup>, H. Inoko<sup>4</sup>, T. Toda<sup>1</sup>.* 1) Div Functional Genomics, Dept Post-Genomics and Diseases, Osaka Univ Grad Sch Med, Osaka, Japan; 2) Dept Clinical Bioinformatics, Grad Sch Med, Univ Tokyo, Tokyo, Japan; 3) Dept Neurology, Grad Sch Med, Univ Tokyo, Tokyo, Japan; 4) Dept Molecular Life Science 2, Sch Med, Tokai Univ, Kanagawa, Japan; 5) Dept Neurology, Kagawa Pref Central Hosp, Kagawa, Japan; 6) Dept Neurology, Sch Med, Juntendo Univ, 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 (alpha-synuclein, parkin, UCH-L1, DJ-1, NR4A2) 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<sup>TM</sup> software and compared the pattern of PCR products of pooled DNAs from patients with PD and controls by the software PickPeak. We have finished to check approximately 20,000 markers on all chromosomes and found associations ( $p < 0.05$ ) in about 6~10% of markers tested on each chromosome. Among them, we found a significant association ( $p = 3.9 \times 10^{-6}$ ) with a marker 479C11 on chromosome 1q where another study showed a linkage. Genes in linkage disequilibrium with these markers may be associated with pathogenesis of PD. We will test these markers with pooled DNA from other patients for second and third screenings.

**Significant association between single-nucleotide polymorphisms in the neuron-specific RNA-binding protein ELAVL4 and Parkinson disease.** *M.A. Nouredine, Y-J. Li, L. Zhang, X.J. Qin, R.W. Walters, M.W. Booze, K. Fujiwara, W.K. Scott, M.A. Pericak-Vance, M.A. Hauser, E.R. Martin, J.M. Vance.* Center for Human Genetics, Duke University Medical Center, Durham, NC.

Parkinson Disease (PD) is a progressive neurological disorder characterized by the loss of dopaminergic neurons in the substantia nigra. The cardinal symptoms of PD are resting tremor, bradykinesia, and loss of postural flexibility. While a small proportion of familial PD cases can be attributed to mutations in certain genes that control macromolecular transport, protein degradation, or components of the cellular machinery, most PD cases are sporadic. There is mounting evidence that neuroinflammation plays a role in the cascade of events leading to nerve cell death. Compared to age-matched controls, PD brains show elevated density of glial cells expressing inflammation-associated factors such as cyclooxygenase-2 (COX-2) and proinflammatory cytokines including, TNF and IL-1. In the nervous system, a large number of genes are regulated post-transcriptionally via the interaction of their mRNAs with specific RNA-binding (Hu) proteins such as the vertebrate homologs of *Drosophila* ELAV (for Embryonic Lethal Abnormal Vision). The mammalian ELAV proteins are known to bind to AU-rich elements (AREs) in the 3'-untranslated region of inflammation-associated factors such as TNF, COX-2, IL-1, IL-6, among several other genes. Recently we have identified a linkage peak (Chr.1p, LOD = 3.41) using age at onset (AAO) of PD as a quantitative trait (Li et al., 2002). The gene ELAVL4 lies in this linkage region. To determine if ELAVL4 is a candidate gene for AAO in PD, we conducted family based association analysis using the Quantitative Transmission Disequilibrium Test (QTDT) on a dataset of 643 families. We found a significant association ( $P=0.01$ ) in one SNP in ELAVL4 (rs#967582). Using the pedigree disequilibrium test (PDT) we also found marginally significant association ( $P=0.04$ ) between another SNP in ELAVL4 (rs#2494876) and PD risk in a subset of early onset families ( $n=70$  families). We are in the process of examining this gene as a potential factor that links AAO, neuroinflammation, and progression of PD.

**A significant evidence for linkage of febrile seizures to chromosome 18p11 and possible association of a common haplotype in the IMPA2 gene in Japanese families.** *J. Nakayama*<sup>1, 2</sup>, *N. Yamamoto*<sup>1</sup>, *K. Hamano*<sup>3</sup>, *N. Iwasaki*<sup>2</sup>, *M. Ohta*<sup>4</sup>, *S. Nakahara*<sup>5</sup>, *A. Matsui*<sup>2</sup>, *E. Noguchi*<sup>1</sup>, *T. Arinami*<sup>1</sup>. 1) Department of Medical Genetics, University of Tsukuba, Ibaraki, Japan; 2) Department of Pediatrics, University of Tsukuba, Ibaraki, Japan; 3) Kitaibaraki Municipal General Hospital, Ibaraki, Japan; 4) Toride Kyodo General Hospital, Ibaraki, Japan; 5) Kensei General Hospital, Ibaraki, Japan.

Febrile seizures (FS) are relatively common and represent most childhood seizures. In the Japanese population, the incidence rate is as high as 7%. It has been recognized that there is a significant genetic component for susceptibility to this type of seizure. Five susceptibility FS loci have been identified on chromosomes 8q13-q21(*FEB1*), 19p(*FEB2*), 2q23-q24(*FEB3*), 5q14-q15(*FEB4*), and 6q22-q24. To find novel susceptibility loci for familial FS, we conducted a genome-wide linkage screening of 48 Japanese nuclear families (190 members) with FS children (59 affected sib-pairs) using 400 microsatellite markers separated by an average distance of 10cM. We used the GENEHUNTER program to performed nonparametric multipoint linkage analysis. Significant linkage was suggested at the marker D18S1158 on chromosome 18p11.2 ( $P = 0.0001$ ). This region includes the *IMPA2* gene, which codes for myo-inositol monophosphatase (IMPase) 2. Inositol phosphatases play a crucial role in the phosphatidylinositol signaling pathway. IMPase converts inositol monophosphates to myo-inositol. IMPase is inhibited by lithium which has proconvulsant effect, and stimulated by carbamazepine, an anticonvulsant. We performed a systematic search for mutations in 24 unrelated Japanese FS patients and detected seven variants (-708G>A, -461C>T, -207T>C, IVS1-15G>A, 159T>C, IVS5+13-14insA, and 558C>T). Haplotype analysis in 59 Japanese families (223 members) with FS revealed an association of a common haplotype in *IMPA2* with FS ( $p = 0.0009$ ). The present study provided preliminary evidence for the *IMPA2* or near locus on chromosome 18p11 as a candidate genomic region for FS.

**Mapping of a novel form of infantile onset epilepsy syndrome to chromosome 2p.** *C. Proukakis<sup>1</sup>, M. Simpson<sup>1</sup>, A. Pryde<sup>1</sup>, M.A. Patton<sup>1</sup>, H. Cross<sup>2</sup>, A.H. Crosby<sup>1</sup>.* 1) Dept Medical Genetics, St George's Hospital Medical School, London, United Kingdom; 2) Dept of Ophthalmology, University of Arizona School of Medicine, 655 N. Alveron Way, Tuscon, USA.

We have identified an autosomal recessive form of severe intractable epilepsy and extreme developmental retardation which typically presents within the first six months of life. The seizure activity consists of both tonic-clonic and partial seizures. Tonic clonic activity is generally controllable through drug therapy, however, partial seizure activity persists. Developmental retardation appears to be slowly progressive, limb movements of affected individuals have an athetoid character and progress with the loss of ability to grasp objects. We ascertained 8 affected individuals with this syndrome from two families which both originate from the same Amish community in Ohio, USA. All 4 parents of these two families have been linked to a fifth generation common ancestor. Using homozygosity mapping assuming a founder effect, we identified a single region of homozygosity on chromosome 2p. Marker saturation analysis indicated that the critical region spans ~5cM equating to ~16Mb and contains approximately 50 known or predicted genes.

**Rare allele in Synapsin 2 (Syn2) associated with decreased rate of schizophrenia and schizoaffective disorder. V.**

*Saviouk*<sup>1</sup>, *E.W.C. Chow*<sup>2</sup>, *A.S. Bassett*<sup>2</sup>, *L.M. Brzustowicz*<sup>1,3</sup>. 1) Dept. of Genetics, Rutgers University, Piscataway, NJ; 2) Dept. of Psychiatry, University of Toronto, and Schizophrenia Research Program, Queen Street Division, Centre for Addiction and Mental Health, Toronto; 3) Dept. of Psychiatry, University of Medicine and Dentistry of NJ, Robert Wood Johnson Medical School, Piscataway.

Synapsins comprise a family of 5 proteins that are associated with cytoplasmic surface of the synaptic vesicles and implicated in synaptogenesis and neurotransmitter release, and in localization of NO synthase in proximity of its targets in presynaptic neurons. A microarray analysis of dorsal prefrontal cortex showed a decrease in transcripts of Syn2 gene (Mirnics et al., 2000). 337 subjects from 24 Canadian families of Celtic origin and 244 subjects from 39 North European families from NIMH collection were genotyped by pyrosequencing at the following locations in Syn2 gene: rs308969 (intron 1), rs308964 (intron 2), rs1530305 (intron2), rs308965 (intron 3), rs931676 (intron 4), rs3817004 (intron 5), rs795009 (intron 7), rs795010 (intron 8), rs795011 (intron 9), rs732863 (intron 10), and rs2289706 (exon 11). rs1530305, rs732863, and rs2289706 were not polymorphic in our samples. 2 groups of SNPs (rs308969, rs308964, rs308965, and rs795009, rs795010, rs795011) were in complete LD and formed 2 haplotype blocks covering introns 1-2-3 and 7-8-9. Analysis with the programs Pseudomarker and Transmit reveal that the intron 5 SNP is associated with schizophrenia/schizoaffective disorder. For the Canadian sample, the results were significant at  $p=0.012$  with Pseudomarker (recessive pseudomarker model) and  $p=0.06$  with Transmit (empirical p-value based on 10,000 bootstrap simulations). For the NIMH sample, the respective p-values were 0.004 and 0.0001, and for combined Canadian/NIMH sample, they were 0.0001 and 0.0004. The rare G allele was significantly more common in healthy subjects than in subjects with schizophrenia. SNP block 7-8-9 also showed slight evidence for association in the combined sample by Pseudomarker analysis ( $p=0.03$ ). Preliminary results suggest that Syn2 may have a rare allele that is protective against schizophrenia.

**Association study of the NEO-PI Five Factor Model of Personality and Chromosome 18q21-23 SNPs by using a Multiplex genotyping method.** *S. Villafuerte*<sup>1</sup>, *J. Chisa*<sup>2</sup>, *S. Srijan*<sup>3</sup>, *L. Gleiberman*<sup>5</sup>, *D. Burke*<sup>2</sup>, *N. Schork*<sup>6</sup>, *A. Weder*<sup>5</sup>, *M. Burmeister*<sup>1,2,3,4</sup>. 1) Mental Health Research Inst; 2) Department of Human Genetics; 3) Neuroscience program; 4) Department of Psychiatry; 5) Department of Internal Medicine, Univ Michigan, Ann Arbor, MI; 6) Department of Psychiatry, University of California at San Diego, La Jolla, CA.

One emerging strategy is the study of risk factors that are correlated with psychiatric disorders. The NEO-PI questionnaire is a reliable and validated measure of the five-factor model of personality. One of these factors, Neuroticism, measures levels of anxiety, depression and vulnerability. Epidemiological studies showed that some genetic factors predispose to both Neuroticism and Major depression. Recently, it has been reported that high Neuroticism appeared to be associated with increases in depression overtime and high Conscientiousness was associated with increases in manic symptoms among bipolar patients. Our laboratory has found a variant in BDNF associated with high scores of Neuroticism, the same variant Sklar and Lander found associated with Bipolar disorder. A genome scan has been completed on a group of 258 families, who have completed the NEO-PI questionnaire, drawn from a population in Tecumseh, Michigan. For the anxiety and depression facets of Neuroticism, two LOD scores higher than 2 were identified on chromosomes 15 and 18q21-23, the later been implicated with Bipolar disorder by others. Therefore, we conducted an association study in a population-based sample by analyzing 11 validated SNPs with a minor allele frequency of 0.2 on chromosome 18q21-23 spanning a region of approximately 28 MB. These 11 SNPs are the first of 26 selected SNPs to evaluate and optimize a multiplex SNP genotyping method where several SNPs can be analyzed simultaneously in one DNA sample. The method is based on simultaneous locus-specific amplification followed by ligation detection reaction. Ligated-oligonucleotides are resolved by gel-size fragmentation. In addition to the individual association, we will be able to describe the extent of linkage disequilibrium and construct haplotypes since we have access to family information.

**Association of *HOXD1* and *GBX2* allelic variants with autism spectrum disorders.** *C.J Stodgell*<sup>1</sup>, *N. Wanek*<sup>2</sup>, *M. O'Bara*<sup>1</sup>, *S.L. Hyman*<sup>3</sup>, *S. Bryson*<sup>4</sup>, *P.M. Rodier*<sup>1</sup>. 1) OB/GYN, University of Rochester, Rochester, NY; 2) Biology, Rochester Institute of Technology, Rochester, NY; 3) Pediatrics, University of Rochester, Rochester, NY; 4) Pediatrics, IWK Health Centre, Dalhousie University, Halifax, Nova Scotia.

Autism spectrum disorders (ASDs) are developmental disorders with an estimated prevalence of 3-6/1,000. Genome scans have suggested that loci on Chr. 2 are linked to ASDs. We tested two candidates located on Chr. 2q for their association with ASDs. *HOXD1* is important in the formation of the abducens nerve, and has been linked to Duane syndrome, a congenital abnormality of eye muscle innervation that has been reported in autism. *GBX2* is another transcription factor which is important in maintenance of the isthmus organizer (the mid/hindbrain division) and formation of cerebellar deep nuclei. Both regions are likely to be important in ASD's neuroanatomical pathology. We identified two SNPs in noncoding regions of *HOXD1*. The first is a G>C SNP 391 bases upstream of the start codon; it met allele frequency expectations and was not in transmission disequilibrium. The second SNP (T>C) is located at base 125 of the intron. Frequencies for the T and C alleles were .72 and .28, respectively. In 60 informative matings, the C-allele was transmitted significantly more often from mother to affected child (McNemar stat = 5.13, p<.03). The G allele of the 3' SNP and the T allele of the intronic SNP were linked 94% of the time. Analysis of *GBX2* revealed a 12 base insert (ACAG ACGG CGGG) 3' of the start codon. The frequency of the allele with the insert was .36 in 345 individuals (178 parents, 124 ASD cases, and 43 sibs making up trios, singletons, and affected and discordant sib pairs.). Using the method of Sun et al. (Am J Epi 150:97-104, 1999), we calculated that the allele without the insert was in disequilibrium with ASD (Z=5.02, p<.000001). These data further support the hypothesis that genes responsible for brainstem and cerebellar formation are important candidates for the etiology of ASDs. (Supported by grants to PMR 1PO1HD35466, a Collaborative Program for Excellence in Autism, and 1RO1HD34969.).

**A new locus for autosomal recessive microcephaly and mental retardation maps to chromosome 1p11.2-21.1 in an Israeli Arab family.** *H. Shilo*<sup>1</sup>, *R. Shenhav*<sup>1</sup>, *R. Pessó*<sup>2</sup>, *G.J. Halpern*<sup>1</sup>, *T. Shohat*<sup>3,5</sup>, *S. Shalev*<sup>4</sup>, *M. Shohat*<sup>1,5</sup>. 1) Department of Medical Genetics, Rabin Medical Center and Felsenstein Medical Research Center, Petah Tikva; 2) Maccabi Health Organization, Central Laboratories, Rehovot; 3) Israel Center for Disease Control, Ministry of Health, Tel Hashomer; 4) Genetics Unit, Ha'emek Medical Center, Afula; 5) Sackler Faculty of Medicine, Tel Aviv University, Israel.

Microcephaly is defined as a head circumference of 3 standard deviations or more below the age and sex related mean. It comprises a heterogeneous group of conditions characterized by failure of normal brain growth. The etiology can be divided into environmental or genetic causes, and it can be congenital or secondary. Five autosomal recessive primary microcephaly loci have been mapped to date: MCPH1 at 8p22-pter, MCPH2 at 19q13.1-13.2, MCPH3 at 9q34, MCPH4 at 15q, and MCPH5 at 1q31. Two genes have been identified - the microcephalin gene associated with MCPH1 and the ASPM gene associated with MCPH5. We report an Israeli Arab consanguineous family with two children who have microcephaly and developmental delay. These children have four double first cousins who are similarly affected. Two additional children, siblings of the cousins, were identified as affected and have since died. The clinical characteristics of the syndrome include microcephaly, spasticity with hyperreflexia in the lower limbs and moderate to severe mental retardation. Auditory evaluation, MRI, metabolic work up and karyotype were normal. No major dysmorphic features were found apart from a low forehead and a prominent and broad nose. We ruled out the previously reported candidate loci and then commenced genomewide screening and homozygosity mapping in this family. We identified a new locus for this autosomal recessive microcephaly mental retardation syndrome on chromosome 1p11.2-21.1 ( $Z_{\max} = 5.46$  at  $\max = 0.01$ ) for the marker AFM197YG1. Further mapping defined the minimum critical region in an interval of 17.8 Mb defined by the markers AFMA297XG9 and D1S495. Identification of the genes implicated in microcephaly will provide insights into the pathogenesis of this condition.

**A Genomic screen reveals evidence for novel SPG loci.** *L. Zhang, M.T. Kloos, S. West, P.C. Gaskell, I.K. Svenson, M.A. Pericak-Vance, D.A. Marchuk, A.E. Ashley-Koch.* Duke University Medical Center, Durham, NC.

Hereditary spastic paraplegia (HSP) is extremely genetically heterogeneous with at least 20 loci having been identified. Genomic screen results from 5 families excluded for mutations in spastin (SPG4) and by linkage analysis to SPG3A, SPG4, SPG6, SPG8, SPG10, SPG12 and SPG13, provided promising evidence for linkage on chromosomes 2, 5, 7, and 13. Two of the linked families exhibited supportive evidence for linkage on chromosome 2. These two families had been excluded for mutations in spastin, and did not provide evidence for linkage at SPG13 (Fontaine et al. 2000) or the alsin gene (ALS2) (Hadano et al. 2001; Yang et al. 2001; Eymard-Pierre et al. 2002). These families appear to represent a new chromosome 2 HSP locus (MaxLod = 2.672 and 1.45). A third family showed evidence for linkage to chromosome 13 (MaxLod = 1.497) in the same region as the recently reported autosomal recessive HSP locus, SPG20 (Patel et al. 2002). In order to determine if this family represents an alternative phenotype and inheritance pattern of the SPG20 locus versus a novel locus, we performed mutation analysis of the SPG20 gene, spartin. We examined all seven reported exons, an alternatively spliced exon, as well as another potential exon causing a putative alternative start site with an upstream methionine that we identified through examination of the EST database. We did not detect a spartin mutation in this family, suggesting that SPG20 is not responsible for the linkage observed in this family. The remaining two families demonstrated support for linkage to chromosomes 5 and 7 (MaxLod = 2.093 and 1.802, respectively), which also appear to be novel SPG linkage regions. Thus, we conclude that additional SPG loci exist further supporting that HSP is an extremely genetically heterogeneous phenotype.

**Further evidence of linkage between adult-onset autosomal dominant leukodystrophy and chromosome 5q23.** A. Simoes Lopes<sup>1</sup>, S. Verreault<sup>2</sup>, J-P. Bouchard<sup>2</sup>, G.A. Rouleau<sup>1</sup>. 1) Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada; 2) Hôpital de l'Enfant-Jésus, Quebec City, Quebec, Canada.

Hereditary leukodystrophies have been rarely reported in adults and are most commonly seen as autosomal or X-linked recessive disorders of infancy or childhood. We have identified and characterized a large French-Canadian family with an adult-onset autosomal dominant leukodystrophy. The clinical picture in 6 examined individuals was marked by autonomic abnormalities, followed by neurological deterioration with upper motor neuron signs and gait disturbance. Imaging studies revealed mild cerebral atrophy and a marked symmetrical loss of white matter particularly in fronto-parietal regions as well as spinal cord atrophy. Genealogical studies allowed us to identify 15 affected individuals over 5 generations and we were able to collect all live members for genetic studies. Previous reports of a single American-Irish family with similar clinical findings localized the disease gene to chromosome 5. Linkage and haplotype analysis of markers in this ADLD locus revealed a maximal LOD score of 4.08 at marker D5S2039 in our family and a haplotype spanning a 10cM/15Mb region flanked by markers D5S2055 and D5S2110. To our knowledge, this is the second family described worldwide with this disorder and our results provide further evidence for the localization of a gene responsible for ADLD on chromosome 5q23.

**Association of polymorphisms in MTHFR and TGF1 in Alzheimer's disease patients.** *R.T. Perry<sup>1</sup>, H. Wiener<sup>1</sup>, L.E. Harrell<sup>1</sup>, D. Blacker<sup>2</sup>, R.E. Tanzi<sup>2</sup>, M. McInnis<sup>3</sup>, S.S. Bassett<sup>3</sup>, R.C.P. Go<sup>1</sup>.* 1) Univ Alabama-Birmingham, Birmingham, AL; 2) Massachusetts General Hospital, Charleston, MA; 3) Johns Hopkins University, Baltimore, MD.

Subjects with cardiovascular risk factors such as atherosclerosis and a history of stroke have an increased risk of both vascular dementia and AD. Increased plasma homocysteine (Hcy) levels are a major independent vascular risk factor and have been reported in AD patients as compared to controls. MTHFR is a key enzyme in the conversion of Hcy to methionine. *In vitro* studies have shown the 677 CT missense mutation (A222V) in MTHFR results in a more thermolabile enzyme with 50% less activity. Although several factors can influence Hcy levels, AD patients homozygous for the T allele have been reported to have higher Hcy levels than controls or nonhomozygous patients. Using family-based association testing, we found a significant association of the T allele to AD under the dominant model in subsets of families in the NIMH sibling data set with at least two affected and one unaffected sibling that were not homozygous for the APOE4 allele ( $p=0.012$ ) and with an age of onset 70 years ( $p=0.025$ ). TGF1 is a multifunctional cytokine with pro- and anti-inflammatory properties and, in particular is a key regulator of the brain's responses to injury and inflammation. A negative regulatory element in the promoter associated with decreased transcription contains a CT substitution at position -509. The T allele is marginally associated with higher transcription activity than the C allele and is associated with higher plasma concentrations in a dose dependent fashion. Confirming other studies, we found a significant association of the T allele to AD in families with at least two affected and one unaffected sibling ( $p=0.007$ ) and, when stratified, for families that were not homozygous for APOE4 ( $p=0.026$ ) and those with an age of onset 50 ( $p=0.007$ ). Interestingly, both genes reside in chromosomal regions detected in the genomic scan we reported on this year. These results support evidence these chromosomal regions may harbor a gene(s) important to AD especially those in inflammatory and atherosclerotic pathways.

**A New *LGII* Mutation in Familial Temporal Lobe Epilepsy with Auditory Auras.** *N.F. Santos<sup>1</sup>, R. Secolin<sup>1</sup>, F.R. Torres<sup>1</sup>, E. Kobayashi<sup>2</sup>, L.A.C. Sardinha<sup>2</sup>, F. Cendes<sup>2</sup>, I. Lopes-Cendes<sup>1</sup>.* 1) Department of Medical Genetics, UNICAMP, Campinas SP, Brazil; 2) Department of Neurology, UNICAMP, Campinas SP, Brazil.

**Purpose:** A locus for familial temporal lobe epilepsy with auditory auras (FTLEAA) was mapped on chromosome (ch) 10q and mutations in the leucine-rich glioma inactivated 1 gene (*LGII*) have been found in affected individuals belonging to different FTLEAA families. The objective of the present study is to investigate genetic linkage and *LGII* mutations in a large Brazilian family segregating FTLEAA. **Methods:** Blood samples were collected from 30 family members for DNA extraction by phenol-chloroform method. For linkage analysis, these individuals were genotyped for 12 microsatellite markers: D10S1644, D10S1687, D10S1765, D10S1753, D10S583, D10S185, D10S574, D10S1680, D10S577, D10S192, D10S566 and D10S187 flanking the *LGII* gene. Two-point and multipoint Lod scores (*Z*) were calculated using MLINK and LINKMAP programs from LINKAGE package. For mutation analysis, we screened the entire coding region of the *LGII* gene by PCR, using primers that flanked intron-exon junctions. Nucleotide sequence for three affected, three non-affected and normal controls were analyzed using MegaBACE 1000 dye-terminators. **Results:** Linkage analysis showed *Z*3.00 for seven markers, with *Z*<sub>max</sub>=6.35 at *q*=0.00 for D10S185. Nucleotide sequencing analysis revealed a VIIS7(-2)A-G splicing site mutation in affected individuals evaluated. Posterior BstN1 restriction enzyme analysis showed this mutation in all affected individuals belonging to this family. No mutation was found in non-affected members and normal controls. **Discussion:** We have found a new mutation for FTLEAA, VIIS7(-2)A-G, that destroys the splicing site upstream of exon 8, possibly leading to an exon skipping and producing a truncated protein. Other hypothesis is the occurrence of alternative splicing in exon 8, modifying the frameshift and resulting in a non functional protein. Supported by: FAPESP and CAPES.

**Further evidence of linkage of Alzheimer's disease on Chromosome 12.** *K.C. Stanton<sup>1</sup>, L. Jiang<sup>1</sup>, N. Schnetz-Boutaud<sup>1</sup>, S.J. Kenealy<sup>1</sup>, W.K. Scott<sup>2</sup>, S. Schmidt<sup>2</sup>, J. Gilbert<sup>2</sup>, G.W. Small<sup>3</sup>, A.M. Saunders<sup>2</sup>, A.D. Roses<sup>2</sup>, D.E. Schmechel<sup>2</sup>, M.A. Pericak-Vance<sup>2</sup>, J.L. Haines<sup>1</sup>.* 1) Vanderbilt University, Nashville, TN; 2) Duke University, Durham, NC; 3) UCLA, Los Angeles, CA.

Alzheimer's disease (AD) is a complex disease that is the leading cause of dementia and affects over 4 million individuals in the United States. Although four genes have been identified that influence both early and late onset AD, these genes explain only part of the genetic etiology.

Several genomic screens for AD indicate a region of linkage on chromosome 12 between 12p13 and 12q22, but consensus identification of a gene remains elusive. To further investigate this linkage, a dataset was assembled from several centers. The total dataset consists of 2131 individuals (1250 affected, 780 unaffected, 101 unknown). Fifteen microsatellite markers were selected to adequately cover the region from 17-91 cM (DeCODE map).

Analysis of the datasets identified several linkage signals, especially when the ApoE-4 allele status was used as a stratifying variable. The most notable signals were seen within families with ApoE-4 alleles, including a lod=1.43 for the entire dataset at D12S1090 (57 cM) under the recessive model, and a lod=2.59 for that same marker in the NIMH sample set.

Ordered Subset Analysis (OSA) rank-orders families by a continuous covariate and finds the subset with maximum evidence for linkage to a particular map of markers. We used mean age-at-onset in each family as the covariate and ranked the means from low to high for all fifteen markers. The lod score at D12S1632 (72 cM) increased from 0.00 to 1.58 for the entire dataset. This increase was significant ( $p=0.02$ ). These data suggest that an earlier onset subgroup of families is responsible for at least one genetic linkage signal on chromosome 12 and that phenotypic subsetting is a useful approach for dissecting the genetics of complex traits.

***p53* codon 72 polymorphism and cancer risk in Li-Fraumeni syndrome.** L. Yip, S.J. Hwang, L.C. Strong, G. Lozano. M. D. Anderson Cancer Center, Houston, TX.

Li-Fraumeni syndrome (LFS) is characterized by a young age at onset of cancers such as sarcomas and tumors of the breast, brain, and adrenal cortex. Approximately 70% of LFS patients are heterozygous carriers of germline *p53* mutations. A polymorphism at codon 72 has been shown to be a modifier of wildtype and mutant *p53* activity. In the current study we examined whether the codon 72 polymorphism contributed to the phenotypic variability seen in LFS germline *p53* mutation carriers.

Study subjects were composed of 60 mutation carriers ascertained from an index childhood sarcoma case or from referred kindreds that have a history of cancer consistent with LFS. *p53* mutations were detected using SSCP, functional, and/or DNA sequencing assays. DNA sequencing then identified carriers with the Arg/Arg and Pro/Pro codon 72 polymorphisms. Carriers with the Arg/Pro polymorphism had additional cDNA subcloning, SSCP, and sequencing to determine the polymorphism within the *p53* mutant allele. Chi-square test and Kaplan-Meier survival analysis were used to calculate the significance of excess cancer cases and of differences in cumulative cancer risk in carriers by the codon 72 polymorphism within the *p53* mutant or wildtype alleles. Results of the study showed a significantly higher risk of developing soft-tissue sarcoma in carriers with the codon 72 Pro polymorphism within the *p53* mutant allele relative to those with the Arg polymorphism (Log-Rank  $\chi^2 = 4.49$ ,  $p = 0.03$ ). The results also showed a marginally higher risk of osteosarcoma for carriers with the codon 72 Pro polymorphism within the *p53* wildtype allele relative to those with the Arg polymorphism (Log-Rank  $\chi^2 = 2.99$ ,  $p = 0.08$ ). However, carriers with the codon 72 polymorphism within the *p53* mutant allele were significantly different by ethnicity ( $\chi^2 = 45.4$ ;  $P < 0.001$ ); Caucasian and Hispanic carriers predominantly had the Arg polymorphism while all African-American carriers had the Pro polymorphism. Our data suggest that the polymorphism at codon 72 of *p53* contributes to the variable phenotype seen in carriers of heterozygous germline *p53* mutations and that further investigations are needed.

**A whole-genome scan for resting heart rate: The HERITAGE Family Study.** *P. An<sup>1</sup>, T. Rice<sup>1</sup>, T. Rankinen<sup>2</sup>, A.S. Leon<sup>3</sup>, J.S. Skinner<sup>4</sup>, J.H. Wilmore<sup>5</sup>, C. Bouchar<sup>d</sup><sup>2</sup>, D.C. Rao<sup>1,6</sup>*. 1) Division of Biostatistics, Washington University School of Medicine, St. Louis, MO; 2) Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA; 3) Division of Kinesiology, University of Minnesota, Minneapolis, MN; 4) Department of Kinesiology, Indiana University, Bloomington, IN; 5) Department of Health and Kinesiology, Texas A&M University, College Station, TX; 6) Departments of Genetics and Psychiatry, Washington University School of Medicine, St. Louis, MO.

Background - Elevated resting heart rate (RHR) is an independent risk factor for cardiovascular disease. Since evidence has accumulated supporting a genetic component for RHR, it is of interest to identify genomic regions that harbor genes underlying RHR variation. Methods and Results - A multipoint variance components linkage scan using 654 markers was performed to search for quantitative trait loci (QTL) that influence RHR at baseline and in response to 20 weeks of endurance training (post-training minus baseline) in 99 white and 127 black families in the HERITAGE Family Study. Potentially interesting regions were revealed in whites including 4q, 10q and 11p for baseline RHR and 1q and 21q for the training response. Additionally, promising regions on 2q and 7q for baseline RHR and 3p for the training response were found in blacks, which did not replicate the regions identified in whites. The linkage evidence on 11p at the sulfonylurea receptor gene (SUR) locus was enhanced (lods from 1.36 to 1.98) in a high-normal BP (at least 135/80 mmHg) subset of white families indicating probable involvement of BP interactions at this locus. Conclusions - Of the genomic regions identified here for baseline RHR and its training response, 11p and 4q are most noteworthy due to replications across subsamples and studies. Future fine mapping and association studies in these regions are warranted.

**The Hepatic lipase (C-514T) polymorphism affects serum HDL cholesterol levels but not associated with coronary artery disease in Koreans.** *E.Y Cho<sup>1,2</sup>, H.Y. Park<sup>1,2</sup>, Y.G. Ko<sup>1,2</sup>, J.H. Lee<sup>1,3</sup>, J.E. Lee<sup>1,2</sup>, S.H. Jee<sup>1,4</sup>, Y. Jang<sup>1,2</sup>.* 1) Cardiovascular Genome Center, College of Medicine, Yonsei University, Seoul, Korea; 2) Research Institute of Cardiovascular disease, Division of Cardiology, Yonsei University Medical Center, Seoul, Korea; 3) Department of Food and Nutrition, College of human Ecology, Yonsei University, Seoul, Korea; 4) Department of Preventive Medicine and public Health, Yonsei University, College of Medicine, Seoul, Korea.

Hepatic lipase(LIPC) is a key enzyme that is involved in HDL cholesterol metabolism. The aims of this study are to investigate the association between the hepatic lipase gene polymorphism, plasma lipid levels and coronary artery disease. Five hundred two healthy control subjects (m:f=183:319) and 214 CAD patients (m:f = 165:49) were included. The subjects who taking the lipid lowering drugs were excluded. Fasting serum concentration of lipid and apolipoprotein were measured and the genotypes of two polymorphic sites (C-514T, V95M/G-A) in the LIPC were determined by SNP-IT assay. The genotype frequency of CC, CT and TT at C-517T was 0.31, 0.51, and 0.18, respectively in control group. The allele frequency of T allele was 0.43. In control group, -514TT group showed the highest mean apoA1 level (CC: 113.826.1, CT: 129.530.8, TT: 133.541.5 P<0.05, ANOVA in male, CC: 126.828.5, CT: 132.427.8, TT: 142.933.7 P<0.05, ANOVA in female). Mean HDL level in -514TT group was significant higher than that of in -514CC group in female group (51.2 vs. 56.5, p<0.05) but the trend was not significant in males. The genotype frequency of GG, GA and AA at codon 1 was 0.48, 0.44, and 0.08, respectively in control group. The A allele of V95M was associated with increasing of HDL cholesterol concentration in male group but not statistically significant. The two different site of genotype frequency in Hepatic lipase gene between CAD and controls was not different in both gender groups. Conclusively, the LIPC -514T polymorphism is common genetic variant in Korean and associated with plasma HDL levels. But this genetic variant was not an independent risk for CAD in our study.

**Genome Wide Scan for blood pressure in an isolated Sardinian founder population.** A. Angius<sup>1,2</sup>, E. Petretto<sup>1,2</sup>, M. Adamo<sup>1</sup>, R. Piras<sup>1</sup>, G. Biino<sup>1,2</sup>, M. Fanciulli<sup>1</sup>, M. Pirastu<sup>1,2</sup>. 1) Inst Population Genetics, CNR,SS, Italy; 2) SharDNA Life Science, CA, Italy.

Within Ogliastro Project, a large survey conducted in an isolated Sardinian population, we present results of an epidemiological study and of a GWS on blood pressure in Talana village (1200 inhabitants). This population may provide significant power to the identification of QTLs due to slow demographic growth, high endogamy and inbreeding, reduced genetic heterogeneity, low number of founders, stable culture and accurate genealogical records. Genotyping was carried out on 875 volunteers by a total of 654 informative markers (average het 0.71, SD=0.09) distributed over the genome (NHLBI Mammalian Genotyping Service). So far 355 individuals have undergone an extensive evaluation comprehensive of physical examination, serological analyses, anthropometrical and blood pressure measurements according to the British Hypertension Society protocol, structured interviews to collect living habits, exposure to most common risk factors, medical and medication history, information about familial disorders. Mean age is 53.19 years (55% females), SBP and DBP are normally distributed ( $p < 0.05$ ) with average of 135.20 and 80.10 mmHg respectively. Regression models were performed to find associated variables accounting for environmental factors. To retain power and information inherent to large pedigrees, all 355 subjects were included in 3 big inbred families (mean size 283, n=850, 55% genotyped). Using SOLAR, a genome-wide linkage analysis by variance components approach was conducted including all significant covariates: sex, age, alcohol assumption and anti-hypertensive therapy for SBP; sex, BMI, total cholesterol, LDL, alcohol assumption and anti-hypertensive therapy for DBP. Heritability of SBP and DBP was 0.21 ( $P=0.019$ ) and 0.25 ( $P=0.005$ ) respectively, indicating that a substantial portion of trait variation is attributable to genetic factors. Suggestive linkage evidence for DBP was found on chromosomes 1 (LOD 2.02) and 9 (LOD 2.28). We observed  $LOD > 1.7$  on chromosomes 2, 4, 6, 10 and 19 for DBP. No significant LOD score for SBP was obtained. Supported by grants from Telethon, Italy No.E1185.

**Systemic biomarkers of vascular inflammation in the NHLBI Framingham Heart Study: Genome scan evidence for susceptibility loci on chromosome 1q.** *J. Dupuis<sup>1</sup>, M.G. Larson<sup>2</sup>, R.S. Vasan<sup>2,3</sup>, J.M. Massaro<sup>1,2</sup>, P.W.F.*

*Wilson<sup>2,3</sup>, I. Lipinska<sup>3</sup>, D. Corey<sup>2</sup>, J.A. Vita<sup>3</sup>, J.F. Keaney<sup>3</sup>, E.J. Benjamin<sup>2,3</sup>.* 1) Department of Biostatistics, Boston University School of Public Health, Boston, MA; 2) NHLBI's Framingham Heart Study, Framingham, MA; 3) Department of Medicine, Boston University School of Medicine, Boston, MA.

Vascular inflammation plays a central role in development of atherosclerosis and inflammatory biomarkers predict several cardiovascular disease (CVD) events. There is evidence that genetic variation in inflammation contributes to susceptibility for CVD. Identifying genes influencing markers of inflammation could improve understanding of genetic determinants of CVD. We conducted linkage analyses in 304 extended families from the Framingham Heart Study of 4 systemic biomarkers of vascular inflammation [C-reactive protein (CRP), monocyte chemoattractant protein-1 (MCP-1), soluble intercellular adhesion molecule-1 (ICAM-1), and interleukin-6 (IL-6)]. There were 1054 individuals in the Offspring cohort with measured inflammatory biomarker levels and genotypes available from a 10 cM genome scan, including 172 sibling pairs, 114 sib trios, and 62 sibships of size 4. Another 506 genotyped individuals without phenotype were used in the analyses. Heritability estimates for inflammation markers ranged from 14% (IL-6) to 42% (MCP-1) after log transforming marker data and adjusting for covariates. Variance-component linkage analyses were performed using Genehunter and p-values were computed by a permutation approach. A region with significant linkage to MCP-1 was identified on chromosome 1 (LOD=4.3 at 185.6 cM; genome-wide  $p < 0.002$ ). The 1-locus support interval includes inflammatory candidate genes such as SELE, SELP (E- and P-selectin) and CRP. Other linkage peaks with LOD scores 2 were identified for MCP-1 on chromosome 1 (LOD=2.8 at 69.8cM) and chromosome 17 (LOD=2.4 at 22cM), and for ICAM-1 on chromosome 1 at 228.8 cM (LOD = 2.0) close to the IL10 gene. Our data suggest multiple genes on chromosome 1 may influence inflammation biomarkers, and these genes could have a major role in development of CVD.

**Genome scan of lipid-related traits in Hispanic and African-American families.** *J.E. Hokanson<sup>1</sup>, C.D. Langefeld<sup>2</sup>, S.R. Beck<sup>2</sup>, B.D. Mitchell<sup>3</sup>, J.M. Norris<sup>1</sup>, D.W. Bowden<sup>2</sup>, S.M. Haffner<sup>4</sup>, M.F. Saad<sup>5</sup>, K.D. Taylor<sup>6</sup>, J.I. Rotter<sup>6</sup>, The IRAS Family Study.* 1) Preventive Medicine & Biometrics, University of Colorado Health Sciences Center, Denver, CO; 2) Wake Forest University School of Medicine, Winston-Salem, NC; 3) University of Maryland School of Medicine, Baltimore, MD; 4) University of Texas Health Sciences Center, San Antonio, TX; 5) University of California, Los Angeles, CA; 6) Cedars-Sinai Medical Center, Los Angeles, CA.

Lipid-related traits are known to promote atherosclerosis. These traits have a significant genetic component with significant genetic pairwise correlations. The current study investigates evidence for linkage of genetic markers to lipid-related traits in the IRAS Family Study. Individuals from 45 Hispanic and 21 African-American families provided blood for DNA analysis and phenotypic data. Genotyping was performed by the NHLBI Mammalian Genotyping Service (Marshfield, WI) with an average intermarker distance of 9 cM. Variance components linkage analyses for QTLs were computed using the SOLAR software package. Evidence for co-localization of lipid-related traits are presented in the table (LOD scores).

Chr/Marker	Apo B	Cholesterol	Triglyceride	LDL
1/D1S1596	2.02	2.31		
8/ATT023	1.64	2.46	1.78	
19/D19S559	2.89			0.96

In addition there was evidence for linkage of Apo B to D17S2196 (LOD=1.54), triglyceride to chr 3:TTTA040 (LOD=1.96) and D13S793 (LOD=1.35), and LDL to D6S2439 (LOD=1.77) and chr 15:GATA50C03 (LOD=1.59). Thus, the strongest evidence for linkage potentially represent co-localization of lipid-related traits to chromosomes 1, 8, and 19.

**TWO PROMOTOR GENETIC VARIATION OF APOLIPOPROTEIN A-1 WAS ASSOCIATED WITH HDL-C AND APOA-1 LEVEL IN KOREANS.** *J.Y. Moon<sup>1</sup>, H. Seo<sup>1</sup>, Y.G. Ko<sup>1,2</sup>, S.M. Kang<sup>1</sup>, N. Chung<sup>1</sup>, W. Shim<sup>1</sup>, S.Y. Cho<sup>1</sup>, Y. Jang<sup>1,2</sup>, H.Y. Park<sup>1,2</sup>, E.Y. Cho<sup>1,2</sup>, J.H. Lee<sup>2,3</sup>, J.E. Lee<sup>2</sup>.* 1) Division of Cardiology, Yonsei University College of Medicine, Seoul, Korea; 2) Cardiovascular Genomic Center, Yonsei University College of Medicine, Seoul, Korea; 3) Department of Food and Nutrition, College of human Ecology, Yonsei University, Seoul, Korea.

The large ethnic differences in the lipid profiles and prevalence of coronary artery disease between Caucasians and Asian peoples may relate to both genetic and environmental differences. In this study, we investigated the role of two polymorphisms of apoA1 gene in lipid profiles and serum apoA1 level in healthy Korean subject. We obtained blood samples from unrelated 417 consecutive subjects (M: F=169:248, mean age 47.2 yrs) who dont have any history of CAD, diabetes and hypertension. The apoA1 genotypes were determined by SNPstream 25KTM System. The plasma apoA1 level was determined by immunoturbidometric analyzer. The genotype frequencies of G-75A were 69%, 26%, and 5% for GG, GA, and AA, respectively. The genotype frequency for XmnI site were 57%, 39%, and 4% for CC, CT, and TT, respectively. The T allele frequency in our study population was higher (27.5%) than that of Caucasian(12-14%). The T allele at XmnI site was significantly associated with low triglycerides ( $p=0.009$ ) and high ApoA1 level ( $p=0.049$ ). The A allele at 75 was also associated with higher plasma HDL-C level ( $p=0.040$ ). Significant linkage disequilibrium between two genetic variations was detected ( $p<0.001$ ). Subjects carrying GG genotype at -75 site and TT genotype at XmnI restriction site (GG/TT) were significant lower HDL-C level and lower apoA1 level compared to the other diplotypes(GG/CT, GA/CT, AA/TT). We concluded that the G-75A polymorphism of apoA1 gene is associated to elevated HDL-C level, and two variants in the promoter region of apoA1 gene had strong linkage disequilibrium in Korean. In contrast to previous reports, our study showed that the T variation at XmnI site is associated the decreased triglyceride level and elevated apoA1, suggesting protective effects of T allele.

**Endothelial nitric oxide synthase gene polymorphism in Fabry disease.** *M. Ziv<sup>1</sup>, R. Schiffmann<sup>2</sup>, G. Altarescu<sup>1</sup>.* 1) Genetic Unit, Shaare Zedek Medical Center, Jerusalem, Israel; 2) Developmental and Metabolic Branch, NINDS, NIH, Bethesda, MD.

**Objective:** To determine the frequency of polymorphisms of endothelial nitric oxide synthase gene (eNOS) among Fabry hemizygotes and ascertain their relationship to markers of disease severity. **Introduction:** The gene encoding eNOS is involved in abnormalities of nitric oxide synthesis that mediate endothelial cell dysfunction, a major feature of Fabry disease. Most Fabry patients have private mutations. It is difficult to predict from the mutation the future development of organ involvement. The purpose of the study was to look for possible correlation between eNOS polymorphisms and markers of disease severity. **Methods:** Genotyping for the 897 GT exon 7 polymorphism of eNOS was performed in 44 adult Fabry hemizygotes. The eNOS genotype results were correlated with Fabry genotype, cardiac echo, ECG parameters, lipid profile, and renal function. The polymorphisms in the eNOS gene were compared using ANOVA (both parametric and non parametric) for the three genotype groups (G/G, G/T and T/T). **Results:** The prevalence of allelic variants of eNOS was: 54% G/G, 39% G/T and 7% T/T. This distribution was similar to that of Caucasian populations previously described. A significant association was found between eNOS polymorphisms and ventricular septal thickness ( $p=0.022$ ), aortic root diameter ( $p=0.029$ ) and left ventricular free wall thickness ( $p=0.009$ ), log rank test. The T/T group had the lowest mean left ventricular free wall thickness ( $8.56 \pm 1.38$  mm), septum ( $8.88 \pm 1.64$  mm) and aortic root diameter ( $28.5 \pm 3.96$  mm) and the T/G group had the highest values in these three parameters. **Conclusions:** These results suggest that the eNOS polymorphisms are distributed similar among Fabry patients compared to Caucasians controls. Among patients with Fabry disease the T/T genotype is associated with a milder cardiac Fabry phenotype, implying that eNOS polymorphisms might serve as a mitigating genetic cardiac modifier in Fabry disease as in other vascular disorders.

**Linkage Analysis of a FCH Dutch Population.** *A. van Nas*<sup>1</sup>, *H. Allayee*<sup>1</sup>, *E. Sobel*<sup>1</sup>, *R.M. Cantor*<sup>1,5</sup>, *K.L. Krass*<sup>2,3</sup>, *M. Groenedijk*<sup>6</sup>, *G.M. Dallinga-Thie*<sup>6</sup>, *T.W. de Bruin*<sup>6</sup>, *A.J. Lusia*<sup>1,2,3,4</sup>. 1) Department of Human Genetics University of California, Los Angeles, California; 2) Department of Medicine University of California, Los Angeles, California; 3) Department of Microbiology and Molecular Genetics University of California, Los Angeles, California; 4) Department of Molecular Biology Institute University of California, Los Angeles, California; 5) Department of Pediatrics University of California, Los Angeles, California; 6) Department of Medicine and the Cardiovascular Research Institute Maastricht, Academic Hospital, Maastricht, the Netherlands.

Familial combined hyperlipidemia (FCH) is a common lipid disorder traditionally characterized by elevated levels of plasma cholesterol and triglycerides that is present in 10% to 20% of patients with premature coronary heart disease. Additionally, increased VLDL secretion has been observed in affected patients which has led to a proposal of redefining FCH; namely to consider affected individuals as hypertriglyceridaemic hyperApoB. Twenty Dutch pedigrees (n=388) classified as affected for ApoB and/or triglycerides were analyzed in a genome scan to determine the chromosomal regions harboring genes contributing to FCH. Parametric single-point, multipoint analysis and non-parametric (LOD=3.96, 2.0, 2.8) suggests linkage of ApoB and/or triglyceride levels to a region on chromosome 16. Notably, the degree of allele sharing around this marker was significantly skewed among siblings. The families were re-typed with markers spanning 50cM, reducing the region of interest to 10cM. These data provide evidence that a chromosome 16 locus, may be contributing to plasma ApoB and/or triglyceride concentrations in humans affected with FCH. The region around this locus has been replicated in several different populations of similar disease traits, including Canadian Indian, Chinese Hans, Indo-Mauritians and Scandinavian as well as being syntenic to the QTL region of a mouse model for combined lipase deficiency (CLD). Further analysis involving fine mapping and association studies of candidate genes in the region is currently in progress, as well as the goal to repeat these linkage results in another FCH population.

**The importance of the BamHI-polymorphism in heparan sulphate proteoglycan 2 (HSPG2) for coronary atherosclerosis.** *S. Schulz<sup>1</sup>, C. Glaeser<sup>1</sup>, D. Rehfeld<sup>1,2</sup>, T. Suess<sup>1,2</sup>, U. Mueller-Werdan<sup>2</sup>, K. Werdan<sup>2</sup>, I. Hansmann<sup>1</sup>.* 1) Inst Human Genetics, Univ Halle, Halle, Germany; 2) Dep Internal Med, Univ Halle, Halle, Germany.

HSPG2 is one of the three major classes of heparin sulphate proteoglycans acting within the cardiovascular system and controlling various aspects of vascular development. It is involved in the lipid metabolism by binding lipoprotein lipase and apolipoprotein B and may therefore be related to vascular disease. Methods: In a case control-study we examined the interactions of the BamHI-polymorphism (PM) of HSPG2, lipid metabolism (LDL, HDL, triglycerides, total cholesterol) and coronary atherosclerosis (CAD). We investigated 353 patients with angiographically confirmed coronary diagnosis depending on their angiographically confirmed coronary and lipid state, 126 patients without any coronary symptoms having an abnormal lipid metabolism (mean age: 51.4y) and 227 CAD patients having a distinct pathological lipid profile (mean age: 50.6y). As controls we examined 300 long-standing healthy blood donors with normal lipid metabolism (mean age: 43y). Results: In order to evaluate the interrelation of the HSPG2 BamHI-PM, lipid metabolism and CAD the genotype frequencies were examined according to a dominant, codominant and recessive genetic model within three proband groups. The analysis of the codominant genetic model showed significantly decreased frequencies of the homozygous mutation-carriers TT among CAD patients (0.02) and patients without coronary afflictions (0.04) compared to healthy blood donors with normal lipid profile (0.07,  $p < 0.032$ ). Based on a T-recessive genetic model a comparison of CAD patients showing the most pathological lipid profile and healthy blood donors revealed a 4.2 times higher risk for TT-carriers of developing an unbalanced lipid metabolism connected with severe coronary atherosclerosis ( $p > 0.006$ ; 95% CI: 1.42-12.4). Conclusions: Depending on the pathological lipid profile the TT-genotype of the HSPG2- PM is significantly less frequent. Beside the pathological lipid profile the angiographically confirmed coronary state seems to be only a minor fact in this association study.

**Hypotrichosis simplex of the scalp is caused by nonsense mutations in the corneodesmosin gene.** *E. Levy-Nissenbaum<sup>1</sup>, R.C. Betz<sup>2</sup>, M. Frydman<sup>1</sup>, M. Simon<sup>3</sup>, B. Goldman<sup>1</sup>, N. Jonca<sup>4</sup>, J. Toribio<sup>5</sup>, S. Cichon<sup>2</sup>, M. Guerrin<sup>3</sup>, G. Serre<sup>3</sup>, M.M. Nothen<sup>2</sup>, E. Pras<sup>1</sup>.* 1) Danek Gartner Institute of Human Genetics; Sheba Medical Center, Tel Hashomer, Israel and Tel Aviv University, Tel Aviv, Israel; 2) Department of Medical Genetics, University of Antwerp, Antwerp, Belgium; 3) Unit of Epidermis Differentiation and Rheumatoid Autoimmunity, FRE2623 CNRS - University of Toulouse III, Toulouse, France; 4) Institute of Human Genetics, University of Bonn, Bonn, Germany; 5) Department of Dermatology, University of Santiago de Compostela, Santiago de Compostela, Spain.

Hypotrichosis simplex of the scalp (HSS) is an autosomal dominant form of non-syndromic alopecia (MIM 146520), characterized by loss of scalp hair in late childhood. We have studied three HSS families of various ethnic origin, all of which showed linkage to chromosome 6p21.3. After reducing the linkage interval to 9.5 Mb, we started to screen candidate genes from the linkage interval preferentially expressed in skin tissue. We identified nonsense mutations in the Corneodesmosin (CDSN) gene in all three HSS families. Full segregation of the mutations was observed in all three families. The mutations were not found in 750 control chromosomes. CDSN, a glycoprotein expressed in the epidermis and inner root sheath (IRS) of hair follicles, is a keratinocyte adhesion molecule. To study the mutations, we examined scalp biopsies using CDSN antibodies. In the patients, biopsies revealed reduced labeling intensity of the stratum granulosum in the epidermis and the IRS of hair follicles. Moreover, we noticed irregularly sized clusters located in ridges of the superficial dermis and at the periphery of hair follicles deeper in the dermis. A dominant negative interaction between the mutant and wild type protein may account for loss of cohesion within the IRS. However, in view of the delayed onset of alopecia, it seems more likely that the observed CDSN aggregates are toxic to the hair follicle cells. Our results underline an important step towards understanding the biological mechanisms underlying scalp-specific hair loss.

**Genome-wide homozygosity mapping and linkage analysis in consanguineous families using 11K SNPs identifies a novel deafness locus.** *C. Rosenow<sup>1</sup>, N. Meyer<sup>2</sup>, R. Mei<sup>1</sup>, S. Dong<sup>1</sup>, R.J.H. Smith<sup>2</sup>.* 1) Affymetrix, 3380 Central Expressway, Santa Clara, CA 95051; 2) Department of Otolaryngology and Interdepartmental PhD Program in Genetics, University of Iowa, Iowa City, IA 52242.

Genome sequences are the foundation for genetic analysis including the identification and mapping of single-nucleotide polymorphisms (SNPs). Association of these polymorphisms with disease phenotypes will enable the development of more effective drugs and improved drug safety (personalized medicine). A novel SNP mapping tool together with an assay that uses a single PCR primer was developed to determine the genotype of more than 11000 SNPs in the human genome. One of the applications for this high-content, high-throughput SNP mapping tool is familial linkage analysis. We have genotyped 3 consanguineous families with deafness symptoms. Hereditary deafness affects 1:2,000 newborns and accounts for greater than 50% of severe-to-profound childhood deafness. The genotyping results from one consanguineous family with 7 members were compared to the analysis results generated using a microsatellite panel. Homozygosity by descent (HBD) analysis using 11K SNPs confirmed one of the previously identified DFNB loci on chromosome 1 and identified a novel locus on chromosome 3. Both regions generated identical lod scores of 2.82 and cover a genomic region of approximately 6 Mb. Using the Online Mendelian Inheritance in Man (OMIM) database we could not identify genes in these regions previously associated with deafness. This finding suggests that digenic inheritance is possible and shows the value of additional genotype information for uncovering undetected linked regions. This novel SNP mapping tool will overcome some of the current limitations for genetic analysis and will enable the generation of high-density genotype maps. This research was supported in part by R01-DC02842 (RJHS).

**A flexible software tool for designing and multiplexing SBE SNP assays for detection by MALDI-TOF.** *P. Boyce<sup>1</sup>, D. Brown<sup>2</sup>, J. Goldstein<sup>1</sup>, D. Witmer<sup>1</sup>, D. Hayden<sup>1</sup>, J. Pettengill<sup>1</sup>, Y. Tsai<sup>1</sup>, E. Pugh<sup>1</sup>, E. Luong<sup>3</sup>, R. Nussbaum<sup>3</sup>, K. Doheny<sup>1</sup>.* 1) Center for Inherited Disease Research, IGM, JHUSOM, Baltimore, MD; 2) University of Waterloo, ON, Canada; 3) IDR, NHGRI, NIH, Bethesda, MD.

We present a flexible software tool that allows a user to choose any number of SNPs and assay them at a chosen plex level. The software allows the user flexibility in design criteria to facilitate performance optimization. Design is based upon standard allelic discrimination by single base extension (SBE) analysis. Method: SNPs are downloaded from public databases in FASTA format, and form the input to the Perl script for PCR and SBE design. User specifiable parameters for PCR primer design include the min, max, and optimum primer size, product size and T<sub>m</sub>, as well as an exclusion region around the SNP. Parameters for SBE primer design are homopolymer, repeat, self-homology, and two base pair extension limits. The Perl script launches Primer3 (Rozen & Skaletsky, 2000), passing it these variables for the design of the PCR primers. SNPs that have PCR primers successfully designed are then automatically processed for SBE primer design. The script outputs files to allow loading of PCR primers and associated Primer3 parameter settings into an Oracle database. The script also outputs a file containing all possible SBE primers designed for each assay. This file is the input for a Python multiplexing script. The variables include plex levels, T<sub>m</sub> for the SBE primers, and minimum separation of SBE product masses. The script chooses panels of markers whose mass peaks should be well separated, and for which there is low probability of hybridization among the different products. The output file contains the panels. Results: We used the default parameters and restricted our panels to 5-plex assays. 4,524 SNPs were queued for PCR design, 69% were returned with successful primers designed. 85% of these successfully designed SBE primers. Multiplexing of the successfully designed assays resulted in 1900 assays. Genotyping these 1900 assays resulted in a median genotype success rate of 75%. This software is available by request.

**A Parallel Algorithm of GENEHUNTER on Multi-Processors.** *Y. Mase<sup>1</sup>, K. Kajitani<sup>1</sup>, N. Kamatani<sup>2</sup>, M.*

*Yanagisawa<sup>3</sup>*. 1) Department of Electronics, Information and Communication Engineering, Waseda University, Tokyo; 2) Institute of Rheumatology, Tokyo Women's Medical University, Tokyo; 3) Department of Information and Computer Science, Waseda University, Tokyo.

GENEHUNTER requires a lot of time to analyze large pedigrees. Our purpose is to decrease the computation time of multipoint linkage analysis with larger pedigrees by parallel processing. In parallel processing, message passing is required to distribute transactions among the processors, connected to each other through LAN. The message delay significantly affects the performance. Conant et al. achieved high performance under Myrinet, the expensive network environment, but their method contains the risk that the performance would suddenly drop under the general network. We propose two parallel processing algorithms and implementation to minimize the performance reduction due to the message delay. One algorithm is concerned about the calculation of inheritance distribution. It is often possible to determine the inheritance by genotypes at marker loci, but it is impossible at uninformative loci and thus the inheritance space becomes quite larger. Inheritance distribution at uninformative loci can be calculated if the forward-backward posterior distribution at adjacent marker loci is known. After this, the calculation of posterior distribution can be done independently by each processor. The other algorithm is concerned about the calculation of score functions. We found the calculation of NPL score by Whittemore et al.'s strategy also takes a long time. This function is calculated for each considerable inheritance, so we have paralleled this part to distribute transactions evenly to each processor. In both part several parallel approach can be considered, and we have adopted the methods which minimize the message delay. We applied our algorithms to data of  $2n-f=18$ , where  $n$  denotes the number of non-founders and  $f$  denotes that of founders. When we used 16 processors, the computation time of our implementation was about 10 times faster than GENEHUNTER. Each processor is Pentium III 1GHz and they are connected with 100Mbps network.

**ldlight: A Fast Haplotype Inference Algorithm for Large-Scale Unphased Diploid Genotype Data based on EM Algorithm and Graph Theoretical Data Structure.** *K. Kajitani<sup>1</sup>, Y. Mase<sup>1</sup>, Y. Ito<sup>1</sup>, R. Sato<sup>1</sup>, N. Kamatani<sup>3</sup>, M. Yanagisawa<sup>2</sup>.* 1) Department of Electronics, Information and Communication Engineering, Waseda University, Tokyo; 2) Department of Information and Computer Science, Waseda University, Tokyo; 3) Institute of Rheumatology, Tokyo Women's Medical University, Tokyo.

Haplotype analysis based on Linkage Disequilibrium (LD) has become an efficient tool for disease-gene discovery. The EM algorithm, one of the most popular haplotype inference methods, is considered to have high accuracy. However, it requires a lot of memory storage and running time to deal with a large number of loci. The EM algorithm needs to store inferred haplotype frequencies for all considerable haplotypes and update all the frequencies in each iteration, therefore time and memory requirement increase exponentially as the number of loci increases. It is impossible to handle haplotypes that consist of more than 30 loci. To solve this problem, explosive growth of considerable haplotypes, we present a new and fast algorithm "ldlight"; to infer diplotype configuration for large-scale unphased diploid genotype data based on the EM algorithm and graph theoretical data structure. The algorithm maintains genotype data on a graph structure (n-partite graph), where n is the number of loci. By representing all haplotypes and their frequencies implicitly on the graph structure, it attained to reduce its computational time from exponential to polynomial. We have implemented this algorithm. As a result, ldlight runs about one thousand times faster at 17 loci, with more than 5 times less storage requirement at 14 loci than an EM-based algorithm. And, nearly one hundred times faster at 50 loci than other algorithms that handle large-scale haplotypes. ldlight runs in at most 4 seconds on 6.6 MByte memory for 100 loci on a Celeron 800MHz PC. As for the accuracy, our algorithm can infer haplotype frequencies almost the same as the EM-based algorithm for studying region on human chromosome 5q31.

**Haplotype Tagging Single Nucleotide Polymorphisms and Association Studies.** *D. Thompson*<sup>1,2</sup>, *D. Stram*<sup>3</sup>, *D. Goldgar*<sup>2</sup>, *J.S. Witte*<sup>2</sup>. 1) CR-UK Genetic Epidemiology Unit, Strangeways Research Laboratory, Cambridge, United Kingdom; 2) Unit of Genetic Epidemiology, International Agency for Cancer Research, Lyon, France; 3) Department of Preventive Medicine, University of South California, Los Angeles, CA.

There is considerable evidence that discrete blocks of low haplotype diversity and high linkage disequilibrium exist within the human genome. Within such blocks, information from some single nucleotide polymorphisms (SNPs) may be redundant; the non-redundant subset of haplotype tagging SNPs (htSNPs) can distinguish the majority of the haplotypes. The genotyping effort of an association study can potentially be reduced by estimating the optimal htSNPs using a small subsample of the study population that have been genotyped for a dense SNP map, and then genotyping just these htSNPs in the remainder of the samples. We investigated by simulation how the size of the subsample affects the power of association studies, and what type of subjects it should include. We used the program tagSNPs, which selects htSNPs to minimise the uncertainty in predicting common haplotypes for individuals with unphased genotype data. We tested for an association between haplotypes and disease with conditional logistic regression, using the expected haplotype dosages in subjects as the independent variables. For a simulated candidate-gene study, an average of 27% of the SNPs were designated as htSNPs. Genotyping as few as 25 unphased individuals to select the htSNPs did not appear to reduce the power of an association study, as compared with using all SNPs. For the disease models considered, selecting htSNPs based on cases, controls, or a mixture of both gave similar results. Several different approaches have been proposed to determine htSNPs, ranging from visual inspection to formal analytical approaches. We used simulations to explore whether the different available approaches identify the same optimal htSNPs. These results suggest that the genotyping effort can be substantially reduced by identifying htSNPs in a small subsample, without significant loss of power.

**Linkage Mapping of Type 2 Diabetes Genes in Multiplex African American Families.** *S.C. Elbein<sup>1,2</sup>, W. Zheng<sup>1</sup>, X. Wang<sup>1</sup>, J.J. Cooper<sup>1</sup>, T. Hale<sup>1</sup>, Z. Zheng<sup>1</sup>, S.J. Hasstedt<sup>3</sup>*. 1) Medicine, Slot LRVA/111J-1, Univ Arkansas for Medical Sci, Little Rock, AR; 2) Central Arkansas Veterans Healthcare System, Little Rock, AR 72205; 3) Dept. of Human Genetics, University of Utah Health Sciences Center, Salt Lake City, UT.

Considerable data support a strong genetic component for type 2 diabetes (T2DM), and several regions have been mapped in Caucasian, Hispanic, and Pima Indian populations. Although the prevalence of T2DM in African Americans is 2-fold higher than Caucasians, only a single scan has been published. We used parametric and nonparametric approaches to test for linkage in 66 African American families comprising 504 individuals, including 224 available affected individuals (mean 3.41.3 per family). A genome-wide scan was conducted using microsatellite markers typed both at CIDR (390 markers) and supplemented by 100 local markers typed on a LICOR sequencer and read by SAGA software to increase density in regions of linkage from previous scans (chromosomes 1, 2, 4, 6, 10, 12, and 18). Of these markers, 41 overlapped between the scans. For overlapping regions, files were prepared using each set of markers and analyzed separately. Pedigree structure was revised to resolve paternity problems after completion of the genome scan, and linkage was analyzed using SimWalkII for nonparametric analysis and dominant and recessive parametric analyses using an age and body mass index determined penetrance function. No significant differences were seen in LOD scores between CIDR data and overlapping laboratory markers. NPL analysis detected regions of  $p < 0.01$  only on chromosome 14. Parametric location scores over 1 were seen on chromosomes 1p, 2p, 4p, 14, and 19. The most striking peak was at 50 cM on chromosome 19, where the dominant lod was 2.5. Regions from other scans, including the published African American scan, were not replicated in this study. Our parametric analysis suggests a possible susceptibility locus on the gene rich region of 19q12, although no obvious candidate genes are present. Work is in progress to place additional markers in putative regions of linkage on chromosomes 1, 2, 14, and 19.

**Localizing a Type 2 Diabetes Susceptibility Gene on Chromosome 1q21-q24 in Utah Caucasians.** *H. Wang<sup>1</sup>, W. Chu<sup>1</sup>, R.L. Craig<sup>1</sup>, S.K. Das<sup>1</sup>, Z. Zhang<sup>1</sup>, S.J. Hasstedt<sup>3</sup>, S.C. Elbein<sup>1,2</sup>*. 1) Dept of Medicine, Univ Arkansas Medical Sci, Little Rock, AR; 2) Central Arkansas Veterans Healthcare System, Little Rock, AR; 3) Department of Genetics, University of Utah Health Sciences Center, Salt Lake City, UT.

We previously mapped type 2 diabetes (T2DM) in extended families from Utah to chromosome 1q21-q24 between 164 cM and 190 cM. A dense linkage map suggested two peaks, with the largest peak (recessive LOD=5; MLS=6) corresponding to the 156 Mb to 159 Mb region. To identify genes in this region, we first collaboratively typed 580 single nucleotide polymorphisms (SNPs) over a 20 Mb region encompassing both linkage peaks in pooled samples from 100 diabetic cases and 100 controls each from Utah, Pima, and Amish family studies. We identified 91 SNPs that were tested in additional assays, of which 14 SNPs were confirmed at  $p < 0.01$ . We designed primers to type 285 total SNPs by Pyrosequencing in the region from 147 Mb to 167 Mb in 384 individual Caucasian samples, including near saturation maps in regions detected in pooled typing. SNPs were selected from a combination of published NCBI SNPs and candidate gene screening. Of these SNPs, 21 were not polymorphic, 52 assays failed, and 67 SNPs are still in progress. From 124 SNPs completed in individual samples, we identified three prominent clusters of associated SNPs ( $p < 0.05$ ). Four SNPs associated with T2DM were located near the PKLR locus (152 Mb; most significant  $p = 0.001$ ), 3 SNPs were located near CASQ1 at 156.9 Mb ( $p = 0.003$ ), and 7 SNPs were located near ATF6 at 158.4 Mb ( $p = 0.0001$ ). The associations found at 156.9 are close to those observed in Amish families, whereas the most significant associations at 158.4 Mb have not been observed in other studies. Associations at 158.4 represented at least two different haplotype blocks and thus are independent observations. Both 156.9 and 158.4 clusters lie under the largest linkage peak in this region for families in the original linkage study that did not carry HNF1 alpha variants. Work is in progress to identify haplotype blocks and to screen genes near the 158.4 cluster, and to extend a dense SNP map across the 156 Mb - 159 Mb region.

**Global distribution of a novel trinucleotide microsatellite polymorphism (ATA)<sub>n</sub> in intron 8 of the SLC11A1 gene and susceptibility to pulmonary Tuberculosis.** A. Awomoyi<sup>1,4</sup>, M. Newport<sup>2</sup>, K. McAdam<sup>3</sup>, S.A. Tishkoff<sup>1</sup>. 1) Dept of Biology, University of Maryland, College Park, MD, USA; 2) Cambridge Institute for Medical Research, Wellcome Trust /MRC Building Addenbrooke's Hospital Hills Road Cambridge, CB2 2XY, UK; 3) MRC Laboratories, Fajara, The Gambia; 4) Dept of Microbiology and Immunology, University of Maryland Baltimore, MD, USA.

Solute carrier family 11 member 1 protein (SLC11A1), formerly called NRAMP1, is a pH dependent proton/divalent cation antiporter involved in macrophage function and is thought to play an important role in susceptibility to inflammatory autoimmune disorders and to certain intracellular pathogens especially Mycobacteria, Leishmania and Salmonella species. We report a novel trinucleotide (ATA)<sub>n</sub> intron 8 polymorphism that was genotyped initially in 485 adult individuals originating from 8 different world populations; Gambia, Nigeria Tanzania, Lebanon, North Europe, Russia China and South America (including 80 Gambian cord blood samples). We compared the range of distribution of alleles of this polymorphism in Gambian cord blood obtained from infants and adult healthy controls and found no significant difference in distribution. Lastly, we determined whether this microsatellite is associated with pulmonary TB by carrying out a TB case/control study in The Gambian population (318 cases and 146 controls). We found no association between this marker and pulmonary TB cases and healthy controls in The Gambian population. This study has identified a novel trinucleotide polymorphism in intron 8 of the SLC11A1 gene in global populations. This will be a useful marker for haplotype analysis and linkage mapping studies of the SLC11A1 region and genetic susceptibility to mycobacterial infection and autoimmune disorders.

**Simple sequence variations and molecular evolution in the WFS1 gene.** *T.A. Sivakumaran<sup>1</sup>, S. Li<sup>2</sup>, J. Zhang<sup>3</sup>, M. Burmeister<sup>2,4,5</sup>, M.M. Lesperance<sup>1</sup>.* 1) Department of Otolaryngology-Head Neck Surgery; 2) Mental Health Research Institute; 3) Ecology and Evolutionary Biology; 4) Psychiatry; 5) Human Genetics, University of Michigan, Ann Arbor, MI.

While mutations in the WFS1 gene are known to cause both recessive Wolfram syndrome type I and dominant low-frequency sensorineural hearing loss, the function of the protein remains unknown. In general, Wolfram syndrome is caused by inactivating mutations, while low-frequency hearing loss is caused by dominant missense mutations in exon 8. WFS1 contains a large number of coding variants, 45% of which are non-synonymous substitutions. Most variants are in exon 8, the largest WFS1 exon. 1812 bp of coding and flanking non-coding regions of exon 8, were sequenced in 105 control individuals with known ethnicity from worldwide populations to determine population-specific sequence variations. 52 variants were identified: 20 were nonsynonymous (5 novel), 27 synonymous, and 5 intronic or small coding deletions. Most variants were rare and population-specific. To gain insight into the function of WFS1, the evolution of the DNA sequence in primates was examined. Sequencing exon 8 in 25 primate species (including great apes, old and new world monkeys, and prosimians) revealed that exon 8 was 90-99% conserved and under strong purifying selection. Although the R allele of H611R is associated with increased suicidality in bipolar patients, no positive selection was seen as both H and R appear to have evolved in parallel in mammals. The alignment of translated sequences including mouse and rat showed that the majority of the non-synonymous varying sites were highly conserved. SIFT analysis correctly predicted 65% of known mutations as deleterious, while the percent of non-synonymous substitutions predicted as tolerant were 63% and 50% for common and rare variants, respectively. The transmembrane, extracellular, and intracellular domains, including the hydrophobic C-terminal domain containing most deafness mutations, show evidence of strong purifying selection. These findings suggest that despite the abundance of non-synonymous variants, these domains hold important functions for the protein that are yet unknown.

**Gene responsible for mitochondrial myopathy and sideroblastic anemia (MSA) maps to chromosome 12q24.33.**

*K. Casas*<sup>1,3</sup>, *Y. Bykhovskaya*<sup>1,3</sup>, *E. Mengesha*<sup>1</sup>, *D. Wang*<sup>1</sup>, *H. Yang*<sup>1</sup>, *K. Taylor*<sup>1</sup>, *A. Inbal*<sup>2</sup>, *N. Fischel-Ghodsian*<sup>1</sup>. 1) Cedars-Sinai Medical Center, Ahmanson Department of Pediatrics, Steven Spielberg Pediatric Research Center, Los Angeles, CA, USA; 2) Institute of Thrombosis and Hemostasis, Sheba Medical Center, Tel Hashomer and Sackler School of Medicine, Tel Aviv University, Israel; 3) Co-first authors.

Mitochondrial myopathy and sideroblastic anemia (MSA) is a rare autosomal recessive disorder of oxidative phosphorylation and iron metabolism. Individuals with MSA present with weakness and anemia in late childhood and may become dependent on blood transfusions. Recently, we reported affected sibling pairs in two related families from a Jewish Iranian kindred living in the U.S. [Casas et al. 2003]. A genome scan and fine mapping of DNA from the two families revealed homozygous alleles in the affected individuals, and a multipoint lod score of 3.3, within 2.3 Mb of chromosome 12q24.33. Previously, [Inbal et al. 1995] described siblings with a similar clinical phenotype who lived in Israel but originated from the same Iranian town as the U.S. families. Focused analysis of DNA from the Israeli family confirmed the presence of identical, homozygous alleles in the affected of all three families within 1.2 Mb of chromosome 12q24.33. Combined multipoint linkage analysis revealed a maximum lod score of 5.41 at the 132 cM position of chromosome 12. Therefore, in three Jewish Iranian families, a disease gene for MSA maps to a 1.2 Mb region of chromosome 12q24.33. This region contains six well described genes (SFRS8, MMP17, ULK1, PUS1, EP400, and GALNT9) and at least fifteen additional putative transcripts. The known genes are expressed in multiple tissues and lack a function specific to mitochondria, making none an obvious candidate. The eventual identification of the disease gene in MSA is expected to provide insight into the tissue specificity and phenotypic variability of mitochondrial disease.

**Linkage of diabetic nephropathy to chromosome 18 in African Americans.** *C.J. Colicigno, B.I. Freedman, M.M. Sale, J.A.E. Dusel, P.J. Hicks, C.D. Langefeld, S.S. Rich, D.W. Bowden.* Center for Human Genomics, Biochemistry, Wake Forest Univ School of Med, Winston Salem, NC.

We have conducted a genome-wide search for linkage to T2DN in 214 affected sib pairs from 171 African American (AA) families (361 total subjects). Among the sibs with DN, 49 had overt DN (serum creatinine concentration > 2 mg/dl or urine albumin:creatinine ratio > 300 mg/g), and 312 had end-stage renal disease (ESRD). Median age of onset of ESRD was 17 + 9.4 years after diagnosis of diabetes. The AA diabetic subjects had a mean + SD (median) age of 59.4 + 9.1 (60) years, mean age at diabetes onset 39.2 + 10.9 (39) years, mean body mass index 30.5 + 6.9 (29.4) kg/m<sup>2</sup>. We tested for genetic linkage between T2DN and 369 polymorphic markers spanning all autosomes using GENEHUNTER-PLUS with ASM (allele sharing model). Genotyping was performed at the Center for Inherited Disease Research.

In our genome scan, strong or suggestive (LOD score > 1.0) evidence for linkage was not detected in the entire set of T2DN affected sib pairs. Subsequent analysis, stratifying on age of onset of diabetes and BMI were performed. Mean age at onset of diabetes was calculated for each sib pair. Analyses were performed by dichotomizing the data into early (below the population median of 39 years) versus late (above the median) age at diabetes onset. In the early age at diabetes-onset sib pairs, the maximum LOD score observed was 2.35 between markers D18S862 and D18S1364, in contrast to a LOD score of 0.00 in the same region for the late age at diabetes-onset group. Stratification for BMI did not reveal additional evidence of linkage.

The 18q22.3-23 region has previously been implicated as harboring type 2 diabetes associated nephropathy (T2DN) susceptibility genes in Turkish and Pima Indian families (Vardarli et al. *Kidney Int* 62:2176-83, 2002). Our results in African Americans replicate evidence for a DN locus in this region. Our results in AAs with T2DN and an early age at diabetes onset suggest that the susceptibility locus in this region may be more frequently involved in progression to ESRD in younger diabetics.

**Genome-Wide Linkage Analysis Identifies Five Candidate Gene Regions in the Cornelia de Lange Syndrome**

**(CdLS).** *L.A. Gillis<sup>1</sup>, T. Strachan<sup>2</sup>, E. Tonkin<sup>2</sup>, M. Kaur<sup>1</sup>, A. Bottani<sup>3</sup>, H.H. Li<sup>4</sup>, L.G. Jackson<sup>5</sup>, M. Devoto<sup>4</sup>, I.D. Krantz<sup>1</sup>.* 1) The Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, PA; 2) University of Newcastle upon Tyne, Newcastle upon Tyne, England; 3) Geneva University Hospital, Geneva, Switzerland; 4) AI duPont Hospital for Children, Wilmington, DE; 5) Drexel University School of Medicine, Philadelphia, PA.

CdLS is a dominantly inherited syndrome characterized by typical facial features, defects of the upper extremities, gastrointestinal dysfunction, cardiac and ophthalmologic abnormalities, growth retardation, and neurodevelopmental delay. Identification of the molecular etiology remains elusive due to: 1) lack of consistent chromosomal rearrangements; 2) paucity of familial cases for linkage analysis; and 3) lack of convincing animal models. Children with dup 3q syndrome have partial phenotypic overlap, and a de novo t(3;17) has been reported in a child with CdLS.

We have identified a unique group of familial cases of CdLS and have performed a genome-wide linkage analysis. Previous haplotype analysis indicated that the dup 3q minimal critical region 3q26-q27 does not appear to be responsible for the CdLS phenotype in 9 families. Genome-wide linkage analysis with 400 fluorescently-labeled polymorphic markers at 10 cM intervals showed in this subset of families a positive lod-score 1.0 in 11 separate chromosomal regions. Linkage for all of these regions was subsequently performed on 4 additional families. Positive lod-scores were obtained in 5 regions: 2q36-2q37 (LOD 2.6); 10p12-10p13 (LOD 2.0); 14q24-14q32 (LOD 1.8); 5p13.2 (LOD 1.6); and 17p13.2 (LOD 1.2). High-resolution (1 Mb) linkage analysis of all positively linked regions is being completed in our 13 families, defining minimal critical regions from which candidate disease genes can be isolated.

**A genome-wide scan identifies novel early onset primary open angle glaucoma loci on 9q22 and 20p12. E.**

*DelBono<sup>1</sup>, J.L. Wiggs<sup>1</sup>, S. Lynch<sup>1</sup>, G. Ynagi<sup>1</sup>, M. Maselli<sup>1</sup>, J. Auguste<sup>1</sup>, J.R. Shi<sup>2</sup>, J.L. Haines<sup>2</sup>.* 1) Dept Ophthalmology, Harvard Medical School/MEEI, Boston, MA; 2) Program in Human Genetics, Vanderbilt University School of Medicine, Nashville, TN.

Glaucoma is a leading cause of blindness world-wide. The disease is characterized by a degeneration of the optic nerve that is usually associated with elevated intraocular pressure. The common form of adult onset primary open angle glaucoma is inherited as a complex trait, while the rarer early onset open angle glaucoma (JOAG) exhibits autosomal dominant inheritance. Approximately 10-20% of cases of JOAG are caused by mutations in the TIGR/Myocilin gene. We have identified 26 pedigrees affected with typical early onset primary open angle glaucoma demonstrating autosomal dominant inheritance. We sequenced the TIGR/Myocilin gene in the proband from each family and found mutations in 8 % of this population. To identify novel genes responsible for JOAG, we used families that did not have TIGR/Myocilin mutations for a genome wide screen. 198 total individuals, (105 affected) were included in this study. In this population glaucoma is defined as age of diagnosis before age 35, IOP greater than 22 mmHg in both eyes, glaucomatous optic nerve damage in both eyes, and visual field loss in at least one eye. 238 microsatellite repeat markers spaced at 10 cM intervals were analyzed for linkage assuming an autosomal dominant model, and allowing for genetic heterogeneity (Hetlods). Markers located in five regions on chromosomes 3, 5, 9, 12 and 20 demonstrated initially interesting results (Hetlod > 1.0). Additional markers located in the regions of interest were used for multipoint analysis which resulted in higher lod scores for the chromosomes 9 (Hetlod 4.1) and 20 (Hetlod 4.0). Haplotype analysis confirmed the multipoint analysis and refined the genetic intervals. These results provide evidence for two new loci for early onset primary open angle glaucoma. Evaluation of candidate genes located within these genomic regions is currently underway.

**Issues encountered in analyses confirming previous reports of linkage for dyslexia phenotypes.** *N. Chapman, R. Igo, W. Raskind, J. Thomson, V. Berninger, E. Wijsman.* Univ.of Washington, Seattle, WA.

We use Markov chain Monte Carlo (MCMC) oligogenic segregation and linkage analysis, in addition to variance components based linkage tests, in order to confirm or refute linkage of dyslexia related phenotypes to several previously reported regions of the genome, in our data set of 119 extended families. Careful analyses of a region on chromosome 15q confirm previous reports of linkage, and emphasize the importance of two issues: 1) map specification in multipoint analyses, and 2) calculation of IBD scores using available software.

Single marker analyses showed evidence of linkage of a reading phenotype to one marker in the region. We then conducted multipoint analyses, using 6 markers covering a 9 cM region (Marshfield map) around the initial signal. Several difficulties were encountered. Over such a distance, the published Marshfield map may not be accurate, and two markers were placed at the same location. Consultation of the deCode map placed a subset of the markers. For markers that were placed on both maps, the estimated interval lengths did not always agree well. These challenges led us to try several maps that were consistent with published maps, and to estimate our own from our data set. Linkage results were sensitive to the map used, and we found that the MCMC signal strength could be increased as much as two-fold by use of a slightly different map.

Components of variance were estimated using SOLAR on IBD scores calculated by both Genehunter 2.1 (GH) and Merlin, which perform exact IBD calculations for single and multiple markers. We discovered that even when it does not explicitly trim people from pedigrees, GH may not report IBD sharing for all pairs. Because SOLAR assumes that any pairs not reported share 0 alleles IBD, naively loading the IBD scores from GH into SOLAR results in an incorrect analysis. Merlin reports exact IBD sharing for all pairs in a family, and is therefore more appropriate. In our data set, using the GH scores resulted in a LOD score that was inflated by as much as 52% over those based on Merlin IBD scores.

**Genome scan of asthma and atopy-associated phenotypes in French EGEA families: What is the impact of sample selection on linkage detection?** *E. Bouzigon, M.H. Dizier, D. Torchard, I. Annesi-Maesano, C. Betard, J. Bousquet, F. Gormand, M. Guilloud-Bataille, J. Just, J. Maccario, R. Matran, F. Neukirch, M.P. Oryszczyn, E. Paty, I. Pin, D. Vervloet, J. Feingold, F. Kauffmann, M. Lathrop, F. Demenais.* INSERM EMI0006, Evry, France.

Genome-wide screens of asthma phenotypes have indicated a few consistent regions of linkage. However, the phenotypes linked to a given region differ across studies. This may be partly due to the mode of ascertainment of family samples, which may select different underlying pathways. To investigate this issue further, a genome-wide search for asthma and atopy-associated phenotypes was conducted in the whole sample of EGEA families with at least one asthmatic subject (295 families) and in a subset selected through two asthmatic sibs (110 families). The following phenotypes were considered: asthma, positive skin prick test response to 11 common allergens [SPT], Multi-RAST Phadiatop, total serum IgE levels and blood eosinophil counts [EOS]. A total of 1317 subjects were genotyped for 396 markers. Linkage was investigated by multipoint model-free methods: Maximum Likelihood Binomial approach for binary traits [asthma, SPT, Multi-RAST] and both Haseman-Elston and variance component methods for quantitative measures [IgE, EOS]. While chromosomal regions potentially linked to SPT (5p15, 13q34, 17q22), Multi-RAST (9q21) and total IgE (2q32, 12p13) were mainly found in the whole sample, linkage to EOS (3q13, 13q22) was detected in the subset having a higher proportion of asthmatics and where a potential linkage to asthma (1p33) was observed. The most significant regions were: 12p13 for IgE (LOD score = 2.07;  $p = 0.001$ ) and 17q22 for SPT (LOD = 1.96;  $p = 0.001$ ) in the total sample and 13q22 for EOS (LOD = 3.38;  $p = 0.00004$ ) in the subset. Our results suggest that the genetic determinants underlying blood eosinophil counts may be more closely related to those underlying asthma than to those predisposing to other atopy phenotypes. Identification of the clinical and epidemiological characteristics of the two analyzed samples, which may account for these results, is currently being investigated.

**A mutation of the myosin heavy chain-7 tail domain results in an autosomal dominant hyaline body myopathy.** *P. Carroll, S.N. Abu-Amero PhD, S. Bohlega FRCPC, S.M. Wakil PhD, R. Al-Omar, B. Lach FRCPC, Y. Al Sayed FRCPC, E.J. Cupler MD, B.F. Meyer PhD.* King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

Hyaline body myopathy (HBM) is a rare congenital myopathy with unique histopathological features. We describe HBM with autosomal dominant inheritance in a three generation Saudi family. Eight of 15 children, their mother and maternal grandmother are affected. Five of nine patients presented with non-progressive, proximal and distal weakness, no bulbar, facial or ocular weakness, but significant wasting and loss of subcutaneous fat. The other four patients showed early, progressive scapuloperoneal weakness with early loss of ambulation. Muscle biopsies showed discrete sub-sarcolemmal hyaline bodies (HB) in approximately 20% of type 1 skeletal muscle fibres. A whole genome scan of approximately 7 cM resolution and linkage analysis with GeneHunterPlus resulted in a maximum LOD score of 3.01 at D14S1280. High-density mapping surrounding the linked locus was performed with 8 additional fluorescently labeled microsatellite markers. Multipoint parametric linkage analysis showed that the linkage region with a maximum LOD score of 3.01 extended from D14S742 to D14S608 with a peak NPL score of 3.75 at D14S608. The myosin heavy chain (MYHC) genes MYH6 and MYH7 map to the region between D14S742 and D14S1280. Sequence analysis of the coding regions of MYH7 revealed an A to T transversion at nucleotide position 25596 (M57965) resulting in a histidine to leucine amino acid change at residue 1904 (H1904L). Sequencing of an additional 130 chromosomes from random normal individuals in the population showed only the wild type A nucleotide at position 25596 (M57965). Pathogenicity of the H1904L mutation most likely results from disruption of MYHC assembly.

Program Nr: 1794 from 2003 ASHG Annual Meeting

**Identifying a new gene for secundum atrial septal defect.** *J.T. Granados<sup>1</sup>, W. Mohl<sup>2</sup>, J.D. Brook<sup>1</sup>*. 1) Institute of Genetics, University of Nottingham, Nottingham; 2) Department of Cardiothoracic Surgery, University of Vienna.

Secundum atrial septal defect (SASD) is a congenital cardiac malformation characterized by an abnormal communication between the right and left atria during postnatal life. Untreated SASD can cause pulmonary overcirculation and hypertension, right heart overload and premature death. As an isolated (non-syndromic) anomaly it occurs as a multifactorial trait, although families in which it behaves as an autosomal dominant disease have been described. In some families, it has been possible to identify chromosomal intervals containing genes responsible for this phenotype. Mohl and Mayr(1) described three unrelated families with secundum atrial septal defect and found no recombination with HLA I haplotypes. The data presented in this poster represent further attempts to correlate this findings to linkage studies on the original families using microsatellite markers located in HLA region.

Reference:1)Mohl W, Mayr WR. Atrial septal defect of the secundum type and HLA. *Tissue Antigens* 10: 121-122, 1977.

**Genome-wide Scan in Multigenerational and Extended Pedigrees Segregating ADHD From a Genetic Isolate. M.**

*Arcos-Burgos<sup>1, 2</sup>, F.X. Castellanos<sup>3, 4</sup>, F. Lopera<sup>2</sup>, D. Pineda<sup>2</sup>, J.D. Palacio<sup>2</sup>, J.L. Rapoport<sup>3</sup>, K. Berg<sup>1</sup>, J. Bailey-Wilson<sup>1</sup>, M. Muenke<sup>1</sup>.* 1) NHGRI/NIH/DHHS, Bethesda, MD; 2) Neurosciences Group, University of Antioquia, Medellín, Colombia; 3) NIMH/NIH/DHHS, Bethesda, MD; 4) NYU Child Study Center, New York, NY.

Attention-deficit/hyperactivity disorder (ADHD) is a persistent syndrome characterized by difficulty in maintaining and paying attention, excessive motor activity, and impulsivity for a given developmental level. Here we present a genome-wide scan using 16 extended and multigenerational families segregating ADHD derived from a genetic isolate, the Paisa community of Antioquia, Colombia. 390 short tandem repeat markers (average spacing 9cM) were scored. Two point analysis using a parametric model revealed LOD scores higher than 2.0 in individual families at chromosomes 8p12 (D8S1477, LOD score = 3.27), 4q13.2 (D42367, LOD score = 2.54), 17q11.2 (D17S799, LOD score = 2.35) and 11q23.3 (D11S1998, LOD score = 2.18). Previous genome-wide scans have implicated regions 17p11 and 11q25, but our suggested regions on chromosomes 8 and 4 implicated in ADHD are novel. Observing LODs this high in individual families by chance is highly improbable based on the eLOD scores obtained when a non-linked marker is simulated in these pedigrees. Therefore, taking these data together with other previous reports, it is reasonable to conclude that these regions may harbor risk genes for ADHD. Additional non-parametric and multipoint analyses of these data are ongoing and will be presented. In order to define the ADHD minimal critical regions and eventually identify genes involved in ADHD, we are now in the process to perform high-resolution mapping in these regions.

**Genetic heterogeneity at the *CRYBB2* locus: negative mutational analysis of a family with dominantly inherited cataract with microcornea linked to the beta-crystallin cluster on chr22q.** *G. Billingsley*<sup>1</sup>, *C.E. Willoughby*<sup>1,2</sup>, *A.E. Shafiq*<sup>3</sup>, *S.B. Kaye*<sup>4</sup>, *A. Chandna*<sup>5</sup>, *E. Héon*<sup>1</sup>. 1) Dept of Ophthalmology and Vision Sciences, Hospital for Sick Children, Toronto, ON, Canada; 2) Dept of Medicine, University of Liverpool, Liverpool, UK; 3) Royal Victoria Infirmary, Newcastle-upon-Tyne, UK; 4) Royal Liverpool University Hospital, Liverpool, UK; 5) Royal Liverpool Children's Hospital, Liverpool, UK.

Chain-terminating mutations in *CRYBB2* and *CRYBB1* on chromosome 22q11.2-q12.2 have been associated with autosomal dominant congenital cataracts. The Q155X mutation in exon 6 of *CRYBB2* (MIM#123620) has marked phenotypic variability producing cerulean, Coppock-like and sutural/cerulean cataracts. A G220X mutation in exon 6 of *CRYBB1* was also reported in a family with pulverulent cataract. We mapped a family with congenital nuclear cataract with microcornea to the beta-crystallin cluster on chr22q11.2. Seventeen members (11 affected and 6 unaffected) of a three generation Caucasian family from the UK had a full ocular assessment to document the phenotype. All affected individuals had congenital cataracts requiring surgery in infancy, and 10 individuals also had microcornea. The affected status (presence of cataracts) was determined before the genetic analysis. A panel of 10 candidate loci was selected for preliminary linkage analysis and genotyping was performed on a Pharmacia automated sequencer. Two-point linkage analysis used the MLINK program of the LINKAGE package v5.2. Haplotype segregation was detected with markers D22S1174, CRYB2-CA, D22S258, and D22S1144 (8.16cM). A maximum LOD score of 2.6 (theta=0) was obtained with intragenic marker CRYB2-CA. Exon 6 of *CRYBB2* and *CRYBB1*, which harbor the published chain-terminating mutations, and the complete coding sequence of *CRYBB2* showed no alterations on direct sequencing. This work further outlines the genetic heterogeneity of congenital cataracts at the 22q locus.

**Identification of a prostate cancer susceptibility locus in 37 Jewish families.** *D.M. Friedrichsen<sup>1</sup>, J.L. Stanford<sup>2</sup>, S.D. Isaacs<sup>3</sup>, M. Janer<sup>4</sup>, B-L. Chang<sup>5</sup>, K. Deutsch<sup>4</sup>, E. Gillanders<sup>6</sup>, S. Kolb<sup>2</sup>, K. Wiley<sup>3</sup>, M.D. Badzioch<sup>7</sup>, S.L. Zheng<sup>5</sup>, P.C. Walsh<sup>3</sup>, G.P. Jarvik<sup>7</sup>, L. Hood<sup>4</sup>, J.M. Trent<sup>6</sup>, W.B. Isaacs<sup>3</sup>, E.A. Ostrander<sup>1</sup>, J. Xu<sup>5</sup>.* 1) Division of Human Biology; 2) Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 3) Dept of Urology, Johns Hopkins Medical Institutions, Baltimore, MD; 4) Institute for Systems Biology, Seattle, WA; 5) Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC; 6) National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 7) Dept of Medicine, Division of Medical Genetics, University of Washington; Seattle, WA.

An estimated 220,900 men will be diagnosed with prostate cancer (PC), and 28,900 deaths will be attributed to the disease in the United States this year. Both epidemiological studies and segregation analyses confirm the existence of a genetic component to PC etiology, with an estimated 5-10% of all PC and 43% of early onset (55 years) disease being attributed to an inherited susceptibility. Statistical evidence to date argues for the existence of at least eight PC susceptibility loci. Together these loci account for the disease in less than half of the analyzed hereditary PC (HPC) families.

Ashkenazi Jews represent a well-defined homogenous population with founder mutations previously identified in other cancer susceptibility genes, i.e. *BRCA1* and *BRCA2*. A combined genome wide scan of 37 Jewish HPC families from FHCRC (17 families) and JHU (20 families) identified a region of suggestive linkage with a NPL *P* value of less than 0.01 for nine consecutive markers covering a 40.9 cM region. The maximum NPL score was 2.97 (*P* = 0.002) with a corresponding Kong and Cox Lod score of 3.18 (*P* = 0.00007). Additionally, the maximum multipoint Lod score using a dominant 11-liability class model was 2.47. Analysis is underway to identify recombinants as well as to search for an ancestral shared haplotype. These studies highlight the utility of analyzing defined sets of families with a common origin for reducing locus heterogeneity problems associated with studying complex traits.

**Investigation for the linkage to the 14q13-21 region in 15 French-Canadian families with Restless Legs**

**Syndrom.** A. Levchenko<sup>1</sup>, L. Xiong<sup>1</sup>, A. Desautels<sup>2,3</sup>, J.-B. Riviere<sup>1</sup>, G. Turecki<sup>2</sup>, J.-Y. Montplaisir<sup>3</sup>, G.A. Rouleau<sup>1</sup>. 1) Neurosciences, Montreal General Hospital Research Institute, Montreal, Quebec, Canada; 2) Psychiatry, Douglas Hospital, Montreal, Quebec, Canada; 3) Psychiatry, Sacre-Coeur Hospital, Montreal, Quebec, Canada.

1. Purpose of the study: Restless Legs Syndrome (RLS) is a common sensory-motor disorder characterized by paresthesia of the lower limbs associated with an imperative urge to move. It affects between 5 to 10% of the general population. Many studies have confirmed that a significant fraction of RLS cases are familial. Recently, a novel RLS locus on 14q13-21 was reported by Bonati MT et al in Brain 2003;126:1-8. We aim to determine if this locus is responsible for a fraction of familial RLS cases in the French-Canadian population. 2. Methods: Fifteen kindreds segregating RLS, each with at least five affected individuals, were selected for this study. Four of the microsatellite markers used by Bonati and colleagues to define the locus were typed in our kindreds. Two are within the haplotype shared by the affected members of the family they described - D14S301 and D14S266. Two others are the flanking markers for the candidate region, i.e. D14S70 and D14S1068. Two-point LOD scores were calculated in all families by MLINK (LINKAGE software package), applying the same parameters as in Bonati et al. 3. Results: Only one family presented possible linkage to the markers D14S266 and D14S1068, with maximum LOD score of 1.18 at  $\theta = 0$  for both. The estimated maximum LOD score for this family is 1.5. In the majority of the other families, linkage to the markers D14S301 and D14S266 could not be totally excluded because the shared alleles were also the most common in the general population. Given the high frequency of the disorder in the general population, one cannot exclude an involvement of a common allele in etiology of RLS. 4. Conclusion: Further investigation of this locus is being undertaken using additional microsatellite markers within the described region. This will allow either definitive exclusion of the linkage to that locus in the French-Canadian population or a confirmation of the linkage in at least one family.

**A new locus for non-syndromic autosomal dominant congenital cataract on chromosome 19.** *Vanita. Kumar<sup>1</sup>, D. Singh<sup>2</sup>, HC. Hennies<sup>3</sup>, K. Sperling<sup>4</sup>, JR. Singh<sup>1</sup>.* 1) Centre for Genetic Disorders, Guru Nanak Dev University, Amritsar, Punjab, India; 2) Dr. Daljit Singh Eye Hospital, Sheranwala Gate, Amritsar, Punjab, India; 3) Dept. of Molecular Genetics and Gene Mapping Centre, Max-Delbrueck-Centrum Berlin-Buch, Robert-Roessle-Str. 10, 13125, Berlin; 4) Institute of Human Genetics, Charite Campus Virchow-Klinikum, Augustenburger Platz 1, D-13353, Berlin.

Purpose: To localize and identify the responsible gene for non-syndromic autosomal dominant congenital cataract (ADCC) in an Indian family. Methods: Genome-wide linkage analysis using more than 400 highly polymorphic fluorescently labeled microsatellite markers was carried out in an ADCC family having 15 members in 4-generations as affected with sutural cataract. Genotyping was done on automated DNA sequencer MegaBACE 1000 with Genetic Profiler v. 1.5 software. 2-point and multipoint linkage analysis were performed by means of the program packages linkage v.5.2, Genehunter v.2.1 and simwalk2 v.2.82. Results: In a genome-wide search positive 2-point lod scores greater than 3.0 were obtained with markers on chromosome 19 at 19q13.3. 2-point linkage data at this region was further supported by multipoint lod scores and haplotype analysis. Conclusion: The present linkage data maps a new locus for non-syndromic autosomal dominant congenital cataract at 19q13.3. It further supports the genetic heterogeneity observed for ADCC. Mutation screening in candidate genes at 19q13.3 to identify the molecular defect for sutural cataract in present family is in progress.

**Serotonin 2A receptor (HTR2A) gene polymorphisms: association and linkage with schizophrenia and schizoaffective disorder.** *V. Nimgaonkar, H. Mansour, J. Wood, T. Logue, A. Johnson, K. Chowdari.* Departments of Psychiatry and Human Genetics, University of Pittsburgh School of Medicine, Western Psychiatric Institute and Clinic, Pittsburgh, Pennsylvania, USA.

Several studies have documented an association of schizophrenia with T102C (rs6313), an exonic single nucleotide polymorphism (SNP) of HT2A. However, the association has not been consistently documented. Associations with HT2A SNPs have also been reported for bipolar disorder by several groups, including ours (Ranade et al, in press). To our knowledge, an association with schizoaffective disorder has not been reported. To explore this possibility, we have analyzed 332 Caucasian probands with schizophrenia or schizoaffective disorder (DSM IV criteria). Both parents of 152 patients were also available. Cord blood samples from local live births served as unrelated, unscreened controls (n =274). The putative association with T102C may reflect a primary association with a neighboring polymorphism. Therefore, we have investigated eight SNPs, comprising 4 located at HT2A exons and 4 flanking polymorphisms. In view of artifacts introduced by case-control association studies, we have also utilized the Transmission Disequilibrium Test (TDT). In the schizoaffective group, we detected a significant association at SNP rs6314 using the TDT. Associations at the haplotype level were present using both case-control and TDT analyses. They generally coincided with the haplotypes associated in our earlier bipolar samples. Significant associations were detectable in the schizophrenia sample at rs 1360020 and with related haplotypes using case-control comparisons. Significant TDT results were not observed for the schizophrenia sample. We suggest that associations with HT2A are present across the spectrum of psychoses, but the associated SNPs may vary. This may help explain the discrepancies in the prior studies.

**Haplotype Analysis in Autosomal Recessive Polycystic Kidney Disease (ARPKD) Families and The Determination of Allele Frequencies of Used Markers in The Turkish Population.** *S. Kantarci*<sup>1, 2</sup>, *M.Y. Apak*<sup>3</sup>, *S. Eraslan*<sup>2</sup>, *A. Nayir*<sup>4</sup>, *H. Kayserili*<sup>3</sup>, *B. Kirdar*<sup>5</sup>. 1) Cancer Genome Research Ctr, Cold Spring Harbor Lab, Woodbury, NY; 2) Division of Molecular Genetics, Düzen Laboratories, Istanbul, Turkey; 3) Dpt. of School Medical Genetics, Istanbul University, Istanbul, Turkey; 4) Dpt. of Pediatric Nephrology, Istanbul University, Istanbul, Turkey; 5) Dpt. of Chemical Engineering, Bogazici University, Istanbul, Turkey.

Autosomal recessive polycystic kidney disease (ARPKD) is one of the most important inherited renal diseases with effects on both kidney and liver and causes morbidity and mortality in childhood. The responsible gene, PKHD1, was localized to a 1 cM genetic interval flanked by the D6S1714 (telomeric) and D6S1024 (centromeric) markers on chromosome 6p21.1-p12. Recently, the gene which extends over 469 kb, has been identified. The mutations which cause ARPKD have been found to be scattered throughout the genes 67 exons without evidence of clustering at specific sites. To determine the frequencies of commonly used markers in the Turkish population, we analyzed 200 unrelated chromosomes in the normal population. D6S465, D6S1714, D6S1024 and D6S466 have been identified to have 7, 9, 5 and 9 alleles with a maximum heterozygosity frequency of 0.5900, 0.600, 0.6900 and 0.6900 respectively. Our studies included 20 families with a total of 23 children with ARPKD. Parental consanguinity was present in 12 families. All families analyzed were informative for at least one marker with the exception of the paternal haplotype in one family. Recombination events were detected between D6S1024 and D6S466 in 4 families. A total of 3 prenatal analyses have been performed. Prenatal diagnosis using fetal sonography is of poor reliability, especially in early pregnancy. Haplotype based analysis for prenatal diagnosis is feasible and reliable method in ARPKD families with an unknown mutation.

**Association between asthma in the Japanese population and genes around TNF gene.** *O. Migita*<sup>1</sup>, *E. Noguchi*<sup>1</sup>, *M. Shibasaki*<sup>2</sup>, *K. Ichikawa*<sup>3, 4</sup>, *A. Matsui*<sup>3</sup>, *T. Arinami*<sup>1</sup>. 1) Department of Medical Genetics, University of Tsukuba, Tsukuba city, Ibaraki, JAPAN; 2) Department of Pediatrics, Tsukuba College of Technology, Tsukuba city, Ibaraki, JAPAN; 3) Department of Pediatrics, University of Tsukuba, Tsukuba city, Ibaraki, JAPAN; 4) Department of Pediatrics, Tsukuba Medical Center Hospital, Tsukuba city, Ibaraki, JAPAN.

Tumor necrosis factor (TNF) is a proinflammatory cytokine that participates in the inflammatory reaction in patients with asthma and located within the region encoding the human major histocompatibility complex on chromosome 6p21.3, which showed linkage to atopic asthma in several genome-wide searches for asthma. Previously, we reported that TNF or nearby gene(s) may contribute to the development of asthma. We screened for mutations in the coding regions and promoter regions of BAT1, BAT2, LTA and LTB genes, which is located around TNF gene, in 16 Japanese asthmatics. To determine whether polymorphisms in these genes are associated with the development of asthma, we performed transmission disequilibrium tests of families identified through children with atopic asthma. Genotypes of families were determined by polymerase chain reaction-based restriction fragment length polymorphism, SNaPshot analysis, or direct sequencing method. Transmission disequilibrium tests of 137 asthmatic families revealed that transmission of the -293G allele in the *LTA* and the *LTA*-293G/ *TNF*-1031T/-863C/-857C haplotype to asthma-affected offspring occurred more frequently than expected (-293G allele in the *LTA* gene,  $p = 0.0007$ ; *LTA*293G/*TNF*-1031T/-863C/-857C haplotype,  $p = 0.0001$ ). Our results indicate that the *LTA* 293G/A polymorphism and the *LTA* 293G *TNF* -1031T -863C -857C haplotype may be involved in the development of asthma in the Japanese population.

**Celiac disease is associated to a haplotype on 5q in Scandinavian families.** A.T. Naluai<sup>1</sup>, S. Adamović<sup>1</sup>, A.S. Louka<sup>3</sup>, S. Nilsson<sup>2</sup>, B. Talseth<sup>3</sup>, A.H. Gudjónsdóttir<sup>1</sup>, H. Ascher<sup>1</sup>, J. Ek<sup>4</sup>, L. Samuelsson<sup>1</sup>, L.M. Sollid<sup>3</sup>, J. Wahlström<sup>1</sup>. 1) Clinical Genetics and Pediatrics, Gothenburg, Sweden; 2) Chalmers University of Technology, Gothenburg, Sweden; 3) Inst. of Immunology, Oslo, Norway; 4) Childrens Hospital, Buskerud, Norway.

*Purpose:* To identify the genetic variation or haplotype in our celiac families, that is the underlying cause of linkage to 5q31-33. *Introduction:* Gluten-induced autoimmune disease (GIAD) is among the most common autoimmune diseases in humans. It has features, which make this disease a natural experimental model for other autoimmune diseases.

1. The causative offending agent is known (gluten) and is removable in vivo (by a gluten-free diet).
2. The mandatory HLA genetic component is well known (HLA DQ2-DQ8).
3. The main self-antigen has been recently identified (Human Tissue Transglutaminase ).

*Methods:* We have a well-defined Scandinavian family material consisting of 104 multiplex and 225 simplex families, "trios". Genetic markers are typed using an ABI 3100 Genetic Analyzer and the SNaP-shot kit (ABI). For statistical analysis we use mainly Allegro. *Results:* We obtained strong evidence of linkage to celiac disease (P-value<0.00002) in the region of 5q31-33. A 90% confidence interval has been estimated between markers D5S414 and D5S820 covering 18cM. Starting in the 50% confidence interval (4cM), we have gone further typing a high density of single nucleotide polymorphisms (SNPs). An efficient approach is to combine family-based linkage analysis and association studies. We tested for association in the presence of linkage by the TDT. We located a haplotype, which shows association to the disease (P-value<0.001). Confirmation using various functional genomic approaches will be the next step. Identification of the DNA-variation behind the linkage and association at this locus will give new insight into the cause and development of disease.

**Linkage and association study of allergic asthma with a STAT6 gene polymorphism in a sample of the Italian population.** *G. Malerba<sup>1</sup>, E. Trabetti<sup>1</sup>, R. Galavotti<sup>1</sup>, L. Xumerle<sup>1</sup>, L. Pescollderunn<sup>2</sup>, A.L. Boner<sup>1</sup>, P.F. Pignatti<sup>1</sup>.* 1) Mother-Child & Biol, Genetics, Univ Verona, Verona, Italy; 2) Hospital of Bolzano, Bolzano, Italy.

Several studies reported linkage of chromosome 12 DNA markers with asthma or asthma-associated phenotypes in several populations. Linkage was described either in genome scans or in specific studies of chromosome 12 single regions. Previously we reported linkage of some chromosome 12 DNA markers with allergic asthma in a group of Italian asthmatic families (*Am J Respir Crit Care Med.* 2000. 162: 1587-90). A candidate gene which maps close to the linked region is the Signal Transducer and Activator of Transcription 6 (STAT6) gene. The individuals of the asthmatic families have been genotyped for an intronic biallelic marker of STAT6. The observed frequency of the less frequent allele in the founders was 0.37 and the observed genotype distribution fitted the expected one (Hardy-Weinberg  $\chi^2$ -test=0.07,  $p=0.9$ ). Non parametric linkage analysis did not show a significant value ( $p=0.39$ , simwalk2 program). The transmission disequilibrium test did not show any preferential allele transmission to asthmatic individuals ( $\chi^2$ -test=0.006,  $p=0.9$ , transmit program). These results suggest that STAT6 is not the major susceptibility gene mapping in the previously reported chromosomal region.

**Evidence for an X-linked gene involved in developmental dyslexia.** *F.A. Hol<sup>1,2</sup>, C.G.F. de Kovel<sup>1,2</sup>, J.G.A.M. Heister<sup>1</sup>, J. Willemen<sup>1</sup>, L.A. Sandkuijl<sup>3</sup>, G.W. Padberg<sup>2</sup>.* 1) Department of Human Genetics, University Medical Centre, Nijmegen, The Netherlands; 2) Department of Neurology, University Medical Centre, Nijmegen, The Netherlands; 3) Department Medical Statistics and Bio-Informatics, Leiden University Medical Centre, The Netherlands.

As part of a larger study into the causes of developmental dyslexia, we recruited a three-generation family that reported to have a high proportion of dyslexics among its members. We carried out a genome-wide parametric linkage study to identify the risk locus for this family.

To standardise phenotyping, we administered two reading tests (real words and nonsense words) to all subjects, as well as a so-called verbal competence test. Subjects were labelled dyslexic if they scored low on either one or both reading tests, but also when there was a large discrepancy between reading ability and verbal competence.

Using this compound phenotype we found convincing evidence for a locus on the X-chromosome near marker DXS8043 in Xq27.3 influencing the dyslexia phenotype in this family (multipoint LOD 3.68). We consider this locus co-dominant rather than recessive. In the four carrier males, the risk haplotype was associated with very low reading ability. Although designated affected according to our criteria the phenotype in the nine carrier females was much more variable.

Two key recombinants allowed us to narrow down the region to an 8 cM interval, encompassing band Xq27.3 and small parts of flanking bands. This region contains eight confirmed genes, among which FMR1, the fragile X mental retardation gene, and about 30 predicted genes.

Analysis of 66 affected male sib-pairs and their mothers showed no evidence for contribution of this locus to the risk for dyslexia in the general Dutch population.

**Fine mapping of 10q and 18q for Familial Alzheimer Disease among Caribbean Hispanics.** *J.H. Lee<sup>1,2,3</sup>, R. Mayeux<sup>1,2,3,4</sup>, D. Mayo<sup>5</sup>, V. Santana<sup>2</sup>, J. Williamson<sup>1</sup>, A. Flaquer<sup>1</sup>, A. Ciappa<sup>6</sup>, H. Rondon<sup>7</sup>, P. Estevez<sup>2</sup>, R. Lantigua<sup>8</sup>, M. Medrano<sup>9</sup>, M. Torres<sup>7</sup>, Y. Stern<sup>1,2,4</sup>, B. Tycko<sup>1,6</sup>, J.A. Knowles<sup>5</sup>.* 1) Taub Inst on Alzheimer's Disease and the Aging Brain; 2) Sergievsky Center; 3) Dept of Epidemiology; 4) Dept of Neurology; 5) Columbia Genome Center; 6) Dept of Pathology; 7) Plaza de la Salud Hospital, Santo Domingo, DR; 8) Dept of Medicine; 9) Universidad Tecnologica de Santiago, Santiago, DR.

The genetic basis of familial Alzheimer disease (AD [MIM 104300]) has been a focus of intense investigation, primarily in families of Europeans and Americans of European origin. Although late Alzheimer disease has been linked to regions on chromosomes 10q and 12p, no gene has been identified. Using a unique cohort of Caribbean Hispanic families, we screened the genome using 340 markers on 490 family members from 96 families with predominantly late-onset Alzheimer disease. We observed the strongest support for linkage on 18q (LOD=3.14). However, 17 additional markers (chromosomes 1, 2, 3, 4, 5, 6, 8, 10, 12, and 14) exceeded a two-point LOD score of 1.0 under the affecteds-only autosomal dominant model or affected sibpair models. Based on these results and those of other investigators, fine mapping efforts subsequently focused on the to candidate regions 10q, 12p and 18q. We previously reported the fine mapping effort on 12p showing modest evidence of linkage. To fine map 10q and 18q, we used 521 family members from 104 Caribbean Hispanic families. With additional markers, the evidence for linkage at 10q (134-138 cM) decreased from a NPL score of 3.15 ( $p=0.000486$ ) to 2.1 ( $p=0.0218$ ), but two broad peaks remained overlapping with previously reported peaks. In contrast, the evidence for linkage for 18q strengthened near 107 cM with additional markers, where the 2-point LOD score for D18S541 was 3.37 and the NPL score was 3.68 ( $p=0.000159$ ). This narrow region contains a small number of genes expressed in brain. Our results provide modest support for linkage on 10q in this cohort of Caribbean Hispanic families with familial Alzheimer disease, and strong evidence for a new locus on 18q.

**Linkage Study of Familial Mesial Temporal Lobe Epilepsy: a Progress Report.** *C.V. Maurer-Morelli<sup>1</sup>, N.F. Santos<sup>1</sup>, R. Secolin<sup>1</sup>, R.B. Marchesini<sup>1</sup>, E. Kobayashi<sup>2</sup>, F. Cendes<sup>2</sup>, I. Lopes-Cendes<sup>1</sup>.* 1) Department of Medical Genetics, UNICAMP, Campinas, Sao Paulo, Brazil; 2) Department of Neurology, UNICAMP, Campinas, Sao Paulo, Brazil.

**Purpose:** Recently, we have described a large group of families segregating familial mesial temporal lobe epilepsy (FMTLE). Pedigrees analysis suggest an autosomal dominant inheritance. The objectives of this study is to carry out linkage studies in FMTLE. **Methods:** We studied 53 unrelated families segregating FMTLE. Among these families we have identified two kindreds that are significantly informative for linkage analysis: F-10 and F-26. A total of 57 individuals, including 32 patients were analyzed in this study. DNA was extracted from blood samples from all available families members. A total of 297 polymorphic dinucleotide repeat markers were chosen, using a polymorphism information content (PIC) 0.75%. The screening was performed by PCR amplification. Two-point lod scores (Z) were calculated for each family separately using the LINKAGE package. The region is considered excluded when Z -2 and Z 3 indicates positive linkage. **Results:** Simulation analysis for the two kindreds (F-10 and F-26) indicate a Z<sub>max</sub> of 8.9, 4.5 and 4.4 respectively. To date, we have genotyped 188 markers. The two Brazilian families segregating FMTLE had significant negative lod score for 129 markers, ranging from -2.00 to -10.87 at different recombination fractions. However, 59 markers were non-informative with lod-scores ranging from -1.98 to 1.91 at different recombination fractions. **Conclusion:** By linkage studies we can confirm or exclude genetic linkage between selected markers and disease *loci*. The genotyped markers covered 63% of the genome and significantly excluded about 44% of the human genome. Although the two families selected for the initial study are very informative for linkage, we had 31% of non-informative results with the highly polymorphic markers tested. This results emphasizes the high risk of a genome-wide search by linkage analysis, even in the presence of a good family material. Supported by FAPESP.

**A Second-generation genome scan for human obesity reveals linkage on 12q23-24.** *WD. Li<sup>1</sup>, C. Dong<sup>1</sup>, D. Li<sup>1</sup>, H. Zhao<sup>2</sup>, R.A. Price<sup>1</sup>.* 1) Dept Psychiatry, BehavioralGen, Univ Pennsylvania Medical Ctr, Philadelphia, PA; 2) Departments of Epidemiology and Public Health, Yale University, School of Medicine, New Haven, CT.

A total of 30 genome scans (including our first genome scan) have been completed for obesity and related phenotypes, with more than 150 reports of linkage to more than 70 regions. However, many reports were based on small sample sizes, and few of the many reports reached genome-wide levels of significance. Here we report significant linkage from a scan of a large sample segregating extreme obesity and normal weight. We have used 382 microsatellite markers in 1,297 individuals from 260 European American families. We conducted nonparametric linkage (NPL) analyses for dichotomous body mass index (BMI, using BMI27kg/m<sup>2</sup>, BMI30kg/m<sup>2</sup>, BMI35kg/m<sup>2</sup>, and BMI40kg/m<sup>2</sup>) using GENEHUNTER. We also analyzed quantitative traits (BMI, %fat, and waist circumference) by the family regression method using MERLIN\_regress. We found evidence for linkage on chromosome 12 (125 cM, D12S2070, LOD=3.79, p=0.00001 for %fat; LOD=2.98, p=0.0001 for BMI; LOD=2.86, p=0.00014 for waist circumference), chromosome 21 (58 cM, D21S1446, LOD=4.27, p=0.00001 for %fat), and chromosome 13 (83 cM, D13S779, LOD=2.82, p=0.0002 for BMI, LOD=1.80, p=0.002 for waist circumference) by family regression analyses. We also obtained suggestive linkages on chromosomes 3, 7, 8, 9, 12, and 13 for discrete BMI by GENEHUNTER. Adding 3 additional markers to the intervals flanking the chromosome 12 peak yielded a LOD score of 4.08 (p=0.00001) for %fat at 116 cM and LOD scores of 3.57 (p=0.00003) and 3.05 (p=0.00009) for BMI and waist circumferences, respectively, at 125 cM. Our results suggest multiple loci that could influence obesity; particularly a locus in chromosome region 12q23-24.

**Genome Scan for Loci in Multiplex Nonsyndromic Cleft Lip Families from Colombia and Ohio.** *L. Moreno<sup>1</sup>, M. Arcos-Burgos<sup>2</sup>, M. Marazita<sup>3,4</sup>, M.E. Cooper<sup>4</sup>, T. Goldstein<sup>4</sup>, K. Krahn<sup>1</sup>, C. Valencia<sup>5</sup>, A.C. Lidral<sup>1</sup>.* 1) Dept. of Orthodontics and Dows Institute of Dental Research, Univ. of Iowa, Iowa City, IA; 2) Dept. of Biology, Univ. of Antioquia, Medellin, Colombia; 3) Center for Craniofacial and Dental Genetics Division of Oral Biology, School of Dental Medicine Univ. of Pittsburgh, PA; 4) Dept. of Human Genetics School of Public Health, Univ. of Pittsburgh, PA; 5) College of Dentistry, Univ. of Antioquia, Medellin, Colombia, SA.

Introduction: Nonsyndromic cleft lip with or without palate (CL/P) is a common, genetically complex birth defect. Previous human studies are contradictory, suggesting that population heterogeneity may exist. To date two genome-wide scans for CL/P have been reported revealing evidence for linkage to over 12 different loci (Prescott et al. 2000, Marazita et al., 2002). The purpose of this study is to complete a 10 cM genome-wide scan in 48 families from Colombia-SA and 9 families from Ohio. Methods: Single point (MLINK) and multipoint (SIMWALK) linkage analyses were performed using parametric dominant and recessive models. Results: Preliminary results in the Colombian and Ohio data set showed evidence of linkage for the following loci:

COLOMBIA	3q27	9q22	14q23	15p12	19q13			
MLS	2.99	2.94	2.29	2.78	1.72			
OHIO	1p22	3p14	9q33	10q25	12q24	14q11	17q11	18q12
MLS	3.52	1.73	1.6	1.52	2.36	1.76	1.77	1.23

Conclusions: 7 loci previously identified as having a role in CL/P have been replicated in this study. Furthermore, 6 new loci have been identified confirming population heterogeneity. Efforts are ongoing to complete nonparametric linkage and association analyses in both data sets.

**A Genome-scan for bipolar susceptibility loci among Ashkenazi Jewish families.** *V. Lasseter<sup>1</sup>, M.D. Fallin<sup>2</sup>, P.S. Wolyniec<sup>1</sup>, J.A. McGrath<sup>1</sup>, G. Nestadt<sup>1</sup>, KY. Liang<sup>3</sup>, A.E. Pulver<sup>1</sup>.* 1) Dept Psychiatry, Johns Hopkins Univ, Baltimore, MD; 2) Dept of Epidemiology, Johns Hopkins School of Public Health, Baltimore, MD; 3) Dept of Biostatistics, Johns Hopkins School of Public Health, Baltimore, MD.

Previous linkage studies in bipolar disorder have been discouraging due to inconsistent findings, small data sets and weak signals. Many of these inconsistencies are likely due to genetic heterogeneity. With this in mind, we have recruited families of Ashkenazi descent in hopes of reducing heterogeneity between families to increase the potential to detect a linkage signal to a particular locus. We have now completed a 10cM autosomal genome-wide linkage scan (382 markers) in 22 multiplex families with a bipolar I phenotype and an additional set of 19 families with bipolar I and bipolar II individuals (41 total). We present results for both allele-sharing and parametric linkage analyses as implemented in Genehunter V2.0, with genome-wide significance levels calculated using simulation.

**A genome wide scan for hypertriglyceridemia loci in a large Icelandic cohort maps a gene to chromosome 10p.** A. Helgadóttir<sup>1</sup>, B. Thorsson<sup>2</sup>, G. Thorleifsson<sup>1</sup>, G. Sigurdsson<sup>2</sup>, J. Gulcher<sup>1</sup>, V. Gudnason<sup>2</sup>, K. Stefansson<sup>1</sup>. 1) Dept Cardiovascular, Decode Genetics, Reykjavik, Iceland; 2) The Icelandic Heart Association, Reykjavik, Iceland.

Hyperlipidemia is a major risk factor for cardiovascular diseases. It constitutes a group of complex disorders that have largely unknown etiology, except in rare cases of Mendelian disorders such as familial hypercholesterolemia. To examine the complex nature of hyperlipidemia, a large population-based genetics study of hyperlipidemia (i.e., high fasting cholesterol and/or triglycerides) is being conducted in Iceland with the primary goal of identifying susceptibility genes for hyperlipidemia. Here we report the results from a genome wide scan for a large cohort of families with hypertriglyceridemia. Blood samples from over 1600 Icelandic individuals with hypertriglyceridemia (>85th percentile in serum fasting triglycerides), and over 4000 of their first degree relatives, have been collected and genotyped for this study. Comprehensive phenotypic information, including the presence of other known cardiovascular risk factors, fasting serum lipids, body mass index, waist-and hip circumference, bioimpedance, serum glucose, liver function tests and blood pressure measurements were obtained for all participants. An encrypted genealogy database that covers the entire Icelandic nation was used to cluster 1584 hypertriglyceridemia patients into families. A genome wide scan, using over 1000 microsatellite markers, was performed both on the cohort as a whole and after stratifying the patients on covariates such as sex and BMI. A linkage analysis on 212 non-obese (BMI < 30) male patients uncovered a locus with a lod score of 3.7 on chr 10p. In addition, loci suggestive of linkage (i.e., lod scores 2.2-3.5) were observed on chr 3q, 7q, 8q(a), 8q(b), and 17q. We conclude that we have mapped a gene for hypertriglyceridemia on chr10p and identified multiple loci demonstrating suggestive linkage to the various sub-phenotypes of hyperlipidemia.

**Fine mapping of the Schnyder's Crystalline Corneal Dystrophy.** *V.P. Theendakara<sup>1</sup>, G. Tromp<sup>1</sup>, H. Kuivaniemi<sup>1, 2</sup>, P.S. White<sup>5, 6</sup>, S. Panchal<sup>1, 3, 4</sup>, R.S. Winters<sup>5</sup>, P. Riebeling<sup>7</sup>, F. Tost<sup>7</sup>, M. Hoeltzenbein<sup>8</sup>, T. Tervo<sup>9</sup>, J.S. Weiss<sup>3, 4</sup>.* 1) Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI; 2) Department of Surgery, Wayne State University School of Medicine, Detroit, MI; 3) Department of Ophthalmology, Wayne State University School of Medicine, Detroit, MI; 4) Kresge Eye Institute, Wayne State University School of Medicine, Detroit, MI; 5) Division of Oncology, Children's Hospital of Philadelphia, University of Pennsylvania, PA; 6) Department of Pediatrics, University of Pennsylvania, PA; 7) Augenklinik der Universität Greifswald, Germany; 8) Max-Planck-Institut für Molekulare Genetik, Berlin, Germany; 9) Helsinki University Eye Hospital, Helsinki, Finland.

Schnyder's crystalline corneal dystrophy (SCCD) is a rare autosomal dominant eye disease characterized by corneal clouding, and arcus lipoides, crystalline cholesterol deposition in the anterior corneal stroma, or both. The abnormal deposition of cholesterol in the cornea is postulated to result from a local abnormality of lipid metabolism. A genome-wide scan with 2 large Swede-Finn families previously mapped the SCCD locus to 1p34-p36 (Shearman et al. *Hum Mol Genet* 5:1667-1672, 1996). We recruited 9 new families and carried out fine mapping of the region by analyzing additional markers. Somatic cell hybrids of affected individuals were generated to aid in constructing haplotypes. Haplotype analysis based on recombination refined the candidate interval to a 2.9 Mbp region between markers D1S1612 and D1S1635. The region contains many genes relevant to lipid metabolism and those expressed in the anterior segment of the eye, such as ENO1, PGD, PEX14, KIF1B, SSB1, CLSTN1 and DFF1. We plan to investigate these loci as positional candidates for SCCD.

Program Nr: 1813 from 2003 ASHG Annual Meeting

**High density SNP linkage panel for human genetic studies.** *S.H. Shaw, R. Shen, C. McBride, M. Chee, A. Oliphant, M. Hansen.* Illumina, Inc., San Diego, CA.

The use of single nucleotide polymorphisms (SNPs) in genetic studies is advantageous because of their abundance in the genome and the ability to use highly automated, high throughput technologies for genotyping. We have developed a high throughput SNP genotyping system based on miniaturized arrays of beads located on the ends of optical fiber bundles. The throughput of the system is about 1 million genotypes per day, and is scalable to higher levels. The assay is highly multiplexed, and genotyping calls are made automatically. Each call is assigned a quantitative score that reflects quality.

We have constructed a panel of SNPs for genome-wide linkage studies. Loci for the linkage panel were selected based on high minor allele frequency, uniform distribution throughout the genome, assay validation on our genotyping platform, and high confidence in genotyping calls. The linkage panel is comprised of approximately 4600 loci. The average minor allele frequency (MAF) for these SNPs in a Caucasian population was 0.39 with all loci having a MAF >0.10 and >80% of all loci having a MAF >0.30. The mean physical distance between each locus was 624 kb. We have constructed a genetic map using genotype data from a series of 28 CEPH pedigrees (504 meioses). All loci have consistent map order with the physical map (build 33 genome assembly). Of the approximately 4600 loci, 53% mapped to unique positions on the genetic map, and 79% of the unique map intervals were mapped with high confidence (>1000:1). The mean genetic map interval is 1.4 cM. In addition, relative information content was computed using a subset of 8 CEPH reference pedigrees for comparison to other widely used simple tandem repeat (STR) marker panels. The average information content for all autosomes was 0.95 (range: 0.81-0.99) and is higher than the 5- and 10-cM STR marker panels tested (ABI v2.5 5- and 10-cM, Marshfield v10 10-cM). This SNP linkage panel provides rapid, accurate, and highly informative genetic data for genome-wide linkage studies.

**Search for Finnish prostate cancer predisposition loci - Fine-mapping of chromosome 3p25-p26 and 11q14.** A. Rokman<sup>1</sup>, A. Baffoe-Bonnie<sup>2,3</sup>, E. Gillanders<sup>3</sup>, K.D. Gibbs Jr.<sup>3</sup>, T. Ikonen<sup>1</sup>, M.P. Jones<sup>3</sup>, D. Gildea<sup>3</sup>, D. Freas-Lutz<sup>3</sup>, C. Markey<sup>3</sup>, M.P. Matikainen<sup>4</sup>, P.A. Koivisto<sup>5</sup>, T.L.J. Tammela<sup>4</sup>, O.P. Kallioniemi<sup>6</sup>, J. Trent<sup>7</sup>, J.E. Bailey-Wilson<sup>3</sup>, J. Schleutker<sup>1</sup>. 1) Lab. of Cancer Genetics, Inst. of Medical Technology, Univ. of Tampere and Tampere Univ. Hospital, Tampere, Finland; 2) Div. of Population Science, Fox Chase Cancer Center, Philadelphia, PA 19111, USA; 3) National Human Genome Research Inst., National Inst. of Health, Bethesda, MD 20892, USA; 4) Div. of Urology, Tampere Univ. Hospital and Medical School, Univ. of Tampere, Tampere, Finland; 5) Dep. of Clinical Genetics, Tampere Univ. Hospital, Tampere, Finland; 6) Medical Biotechnology Group, VTT- State Technology Research Center, Turku, Finland; 7) Translational Genomics Research Inst., Phoenix, AZ 85004, USA.

Only a few genes have been identified in the search for prostate cancer (PRCA) susceptibility genes probably due to difficulties raised by the late age at onset, heterogeneity and common nature of the disease. Such a complex disease can be more effectively studied in a homogenous population like in Finland. Recently, a genome wide linkage analysis based on ten multiplex Finnish PRCA families using 413 microsatellite markers was conducted. In that study, positivity was not found in the previously published PRCA predisposing loci. Instead, previously undescribed loci on 3p25-p26 and 11q14, seemed to be most important, with corresponding two-point LOD scores of 2.63 (= 0.01) and 2.99 (= 0.0), and non-parametric NPL scores of 2.27 (p = 0.02) and 2.66 (p = 0.008), respectively. In this study we proceeded to the fine-mapping phase using 39 markers (17 for chromosome 3 and 22 for chromosome 11), which covered the critical areas with 0.1-1.0 cM intervals. Six additional families were included in the analyses, making the total number of families 16. All families were defined as having three or more affected members with at least two sampled affected cases. Altogether 238 DNA samples, including 49 affected cases, were genotyped. The preliminary results strengthen the role of these two chromosomal regions in the PRCA predisposition in Finland.

**Coarse face, dysostosis multiplex, progressive laryngeal obstruction, and severe psychomotor retardation - a new type of autosomal recessive storage-like disease maps to chromosome 1q32.3.** *R. Shenhav<sup>1</sup>, V. Drasinover<sup>1,2</sup>, N. Magal<sup>1,2</sup>, A. Raas-Rothschild<sup>3</sup>, L. Basel-Vanagaite<sup>1</sup>, G.J. Halpern<sup>1</sup>, T. Shohat<sup>4</sup>, M. Shohat<sup>1,2,5</sup>.* 1) Medical Genetics, Rabin Med Center, Petah Tikva, Israel; 2) Felsenstein Medical Research Center, Petah Tikva, Israel; 3) Human Genetics, Hadassah University Hospital, Ein Kerem, Jerusalem, Israel; 4) Israel Center for Disease Control, Ministry of Health, Tel Hashomer, Israel; 5) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

We describe an extended multiply consanguineous Israeli Arab family. Two of the couples had together five affected daughters, all of whom had similar clinical features including coarse facies, macroglossia, short stature, dysostosis multiplex, severe psychomotor retardation, and a variable degree of progressive upper airway obstruction. The phenotypic appearance of these children and many of the clinical features suggest a storage disease, although the absence of organomegaly, mucopolysacchariduria, oligosacchariduria and other specific biochemical and ultrastructural findings rules out the known storage diseases. Based on the pedigree it seems that this family represents a new type of storage-like disease transmitted by autosomal recessive inheritance. We performed a genomewide linkage study in this family. Our data provide evidence for the existence of a disease-causing gene locus on chromosome 1q32.3. Analysis of haplotypes and recombination events suggested that the disease locus is flanked by markers D1S213 and D1S413. Analysis of additional markers in this region narrowed down the critical region to ~1.4 Mb between markers D1S2703 and D1S419 and provided evidence of homozygosity with marker D1S2646. ( $Z_{\max} = 4.45$  at  $\alpha_{\max} = 0.001$ ). The underlying pathogenesis of this autosomal recessive storage-like disease is unknown, and it is hoped that identification of the predisposing gene will allow an insight into and better understanding of the fundamental defect and the mechanisms involved.

**Search for quantitative trait loci for bone mineral density and predisposition to osteoporosis by genome wide linkage analysis.** *K. Sol-Church<sup>1</sup>, L.D. Spotila<sup>2</sup>, D.L. Stabley<sup>1</sup>, C. McKay<sup>1</sup>, H.H. Li<sup>1</sup>, K.F. Wang<sup>1</sup>, G.N. Picerno<sup>1</sup>, J. Korkko<sup>3</sup>, D. Prockop<sup>3</sup>, A. Tenenhouse<sup>4</sup>, M. Devoto<sup>1</sup>.* 1) Dept. Biomedical Research, Nemours Children's Clinic, Wilmington DE; 2) Drexel University, Philadelphia PA; 3) Tulane University, New Orleans LA; 4) McGill University, Montreal Canada.

Osteoporosis is a common condition characterized by reduced skeletal strength and increased susceptibility to fracture. The single major risk factor for osteoporosis is low bone mineral density (BMD) and strong evidence exists that genetic factors are in part responsible for an individual's BMD. In our laboratory, we seek to elucidate the underlying genetic mechanisms for low BMD and predisposition to osteoporosis by means of linkage analysis in a whole genome scan in families of individuals with low BMD. A cohort of 42 multiplex Caucasian families selected through a proband with osteoporosis, comprising a total of 254 individuals, was genotyped for microsatellite markers spaced at an average 10 cM density and Mendelian errors were detected by mean of the Pedmanager software. Linkage to femoral neck and spine BMD was analyzed using the SOLAR package that implemented a variance components approach and association was tested by means of the transmission disequilibrium test using the QTDT software. Several loci with positive support for linkage ( $Z_{\max} > 1$ ) were identified on chromosomes 1, 2, 3, 6, 12, 16, 18 and 22. The positive linkage on chromosomes 2 and 6 was observed for both spine and femoral neck BMD, while the other chromosomes were positive for either one or the other of the two traits. The highest lod-scores were observed between markers located in 1pter-p36.2 and femoral neck BMD ( $Z_{\max} = 2.85$  at marker D1S214). Variance components analysis suggested that a substantial fraction of the total genetic variation was explained by this locus. QTDT analysis also suggested the presence of linkage disequilibrium with two microsatellite markers, D1S489 ( $p=0.0012$ ) and D1S2660 ( $p=0.0098$ ), in the same region. These studies will help identify candidate genes included in the region of linkage, which may be used to identify individuals at risk of developing the disease who may benefit from early prevention therapy.

**IL4 receptor alpha Gene: Linkage and Association analysis of five polymorphisms in Italian families with atopic asthma.** *C. Patuzzo<sup>1</sup>, C. Migliaccio<sup>1</sup>, G. Malerba<sup>1</sup>, E. Trabetti<sup>1</sup>, R. Galavotti<sup>1</sup>, L. Pescollderung<sup>2</sup>, A.L. Boner<sup>3</sup>, P.F. Pignatti<sup>1</sup>.* 1) Sect Biol & Genetics, Mother-Child, Biol & Genetics, University of Verona, Verona, Italy; 2) Division of Pediatrics, Hospital of Bolzano, Italy; 3) Sect of Pediatrics, Mother-Child, Biol & Genetics, University of Verona, Verona, Italy.

Although IL4 and its receptor (IL4RA) mediate pivotal pro-inflammatory functions in asthma there are conflicting reports regarding the involvement of IL4RA variants in the pathogenesis of asthma. In this study we present linkage and association analysis of five polymorphisms (I50V, E375A, C406R, S478P, Q551R) of the IL4RA gene in a large series of well characterized individuals from North-East Italy. All the 823 individuals from 182 families with atopic asthmatic children were tested for clinical asthma, total serum IgE level, Skin Prick Test positivity to common aeroallergens, and bronchial hyper responsiveness to methacholine. The five polymorphisms were genotyped with restriction analysis, RG-PCR and ARMS-PCR and the accuracy of detection was checked by direct sequencing. Non-parametric linkage analysis and transmission disequilibrium test (TDT) were performed with the computer programs GENEHUNTER 2.1 and TRANSMIT respectively. We did not observe linkage or transmission disequilibrium in the families for any polymorphism with any of the phenotypes investigated. A multipoint analysis did not show association between any multipoint haplotype and any phenotype. The conflicting reports may be due to interactions with other factors and population differences. In conclusion, the IL4RA gene does not seem to play an important role in genetic predisposition to atopic asthma in the population tested.

**Genomic Screen for adult-onset primary open angle glaucoma: Follow-up studies suggest loci on 14q11 and 15q.**

*J.L. Wiggs<sup>1</sup>, R.R. Allingham<sup>2</sup>, J. Augustine<sup>1</sup>, J.R. Shi<sup>4</sup>, K. LaRocque-Abramson<sup>3</sup>, M. Hauser<sup>3</sup>, E. DelBono<sup>1</sup>, F.L. Graham<sup>3</sup>, M. Pericak-Vance<sup>3</sup>, J.L. Haines<sup>4</sup>.* 1) Dept Ophthalmology, Harvard Medical Sch, MEEI, Boston, MA; 2) Dept Ophthalmology, Duke Medical Center, Durham, NC; 3) Center for Human Genetics, Duke Medical Center, Durham, NC; 4) Program in Human Genetics, Vanderbilt University School of Medicine, Nashville, TN.

Glaucoma is a leading cause of blindness worldwide. The disease is characterized by degeneration of the optic nerve usually associated with elevated intraocular pressure. The most common form of glaucoma is adult onset primary open angle glaucoma which is inherited as a complex trait. We have previously completed an initial genome screen to identify the chromosomal locations of POAG susceptibility genes. The initial study identified 16 chromosomal regions that demonstrated interesting results, and after the addition of a second pedigree set, further evidence for POAG loci on chromosomes 2, 4, 14, 15, 17 and 19 was obtained. To refine these regions we have added to the analysis: a third group of sibling pairs (195 sibling pairs total), unaffected pedigree members to help establish IBD, and additional markers in the regions of interest. We have analyzed the data using four different methods: parametric analysis using an autosomal dominant model and assuming genetic heterogeneity (Hetlods), nonparametric analysis allowing for complex pedigree structure (LOD\*), nonparametric analysis using all sibpairs (MLS) and ordered subset analysis (OSA) stratifying the pedigree set by age of onset. With the current data set, all four approaches provide evidence for POAG susceptibility loci on chromosome 14q11 (Hetlod 2.5 marker D14S264; LOD\* 1.9; MLS 3.2; OSA lod score 3.68) and 15q (Hetlod 1.4 marker GABRB3; LOD\* 2.06; MLS 3.03; OSA lod 3.19). These results suggest that these two loci likely represent a substantial portion of the genetic effect in POAG. Evaluation of candidate genes located within these genomic regions is currently underway.

**Refinement of the Chronic Distal Spinal Muscular Atrophy (Chronic DSMA) locus on chromosome 11q13.3 and evidence of a founder haplotype in the European population.** *L. Viollet<sup>1</sup>, M. Zarhrate<sup>1</sup>, I. Maystadt<sup>1,2,3</sup>, A. Munnich<sup>1</sup>.* 1) INSERM U-393, Hopital Necker, Paris, France; 2) Department of Human Genetics, Catholic University of Louvain, Brussel, Belgium; 3) FNRS, Brussel, Belgium.

Chronic Distal Spinal Muscular Atrophy (Chronic DSMA) is an autosomal recessive neuromuscular disorder characterized by progressive anterior horn cell degeneration, leading to motor weakness and muscular atrophy predominating in the distal part of the limbs. In a previous study, we mapped the disease gene to a 15.16 cM interval on chromosome 11q13-q21, between markers D11S4076 and D11S1321 in a large Arabian pedigree. We report here additional linkage data resulting from the analysis of eleven unrelated European Chronic DSMA families. All patients presented an identical pattern of muscle wasting, beginning in the lower limbs, with onset extending from 6 months to 10 years. For genetic analysis, we used polymorphic markers from the Genethon map and we generated an additional microsatellite marker (called DSM4), located in the 11q13.3 region. A significant linkage (Lod score up to 5 at  $\theta = 0.00$ ) was obtained at the DSM4 locus. A recombination event with the marker locus D11S4136 was observed in two independent inbred families at the centromeric side of the region. At the telomeric side, a recombination event with the marker locus D11S1314 allowed to place the Chronic DSMA gene in a 3 cM interval between loci D11S4136 and D11S1314 on chromosome 11q13.3. In addition, the analysis of markers at loci D11S4184, DSM4 and D11S1369 revealed that the affected individuals harbored a shared haplotype (4-3-2) in 7/11 pedigrees. Interestingly, most of these families originated from Italy or Southern of France. These data suggest that, in the European population, mutant Chronic DSMA chromosomes may derive from a single founder chromosome. The genetic refinement of Chronic DSMA locus allowed us to exclude the IGHMBP2 gene, which mutations have been reported in an early severe DSMA phenotype (SMARD1), and to search for mutations in the candidate genes of the region. Direct sequencing of the coding sequences is currently carried out and will hopefully allow to identify the gene responsible for Chronic DSMA.

**Mapping of an autosomal dominant cataract (ADC) Chilean family to a unique locus.** *L. Richter*<sup>1</sup>, *J.L. Turner*<sup>1</sup>, *F.R. Barra von Bischhoffshausen*<sup>2</sup>, *P. Flodman*<sup>3</sup>, *M.A. Spence*<sup>3</sup>, *J.B. Bateman*<sup>1</sup>. 1) Department of Ophthalmology, Rocky Mountain Lions Eye Institute, University of Colorado, Aurora, CO; 2) Departamento de Oftalmología, Universidad de Concepcion, Concepcion, Chile; 3) Department of Pediatrics, University of California Irvine, Orange, CA.

**Purpose:** To map and identify the gene for ADC in a large Chilean family.

**Methods:** ADC 52 is a four generation Chilean family consisting of 14 individuals with seven affected individuals. Clinically, all the affected patients had the cataract removed except one and the one patient with the lens had a plaque on the posterior capsule of the lens. SIMLINK analysis was used to estimate the power to detect linkage of ADC 52. We have created a panel of markers for the twenty-five known ADC loci based on linkage analysis and identification of gene mutations. We screened the family with our panel using PCR amplifications performed separately for each primer set. The products were resolved on an ABI 373 using Genescan 2.1 software (Applied Biosystems). Two point LOD scores were calculated using LIPED.

**Results:** For a tightly linked marker, we estimated that linkage analysis in the chosen subset of this family will have 70% power to detect a LOD greater than 3; the maximum LOD score achieved over 1000 simulations was 3.25. We calculated LOD scores between the ADC 52 locus and markers on chromosomes 1, 2, 3, 10, 11, 12, 15, 16, 17, 19, 20, 21, and 22 and excluded all loci.

**Conclusions:** Using our ADC screening panel, we excluded linkage in this family with all markers known to be linked to human ADC. Therefore, ADC 52 represents a novel ADC locus. We are conducting a genome-wide scan of this family to determine this locus.

Work supported by NEI Grant 5 R01 EY08282-11.

**Mapping of autosomal dominant retinitis pigmentosa: evidence for a novel disease locus.** *M. Papaioannou<sup>1</sup>, Q. Prescott<sup>1</sup>, R. Koenekoop<sup>2</sup>, M. Loyer<sup>2</sup>, S. Bhattacharya<sup>1</sup>*. 1) Molecular Genetics, Institute of Ophthalmology, UCL, London, UK; 2) McGill Ocular Genetics Laboratory, Montreal Children's Hospital Research Institute, Montreal, Canada.

Retinitis pigmentosa (RP) is a highly heterogeneous group of genetic disorders of the retina that cause night blindness and progressive loss of peripheral vision. RP affects approximately 1:5000 people and can be inherited in an autosomal dominant (adRP), recessive (arRP) or X-linked (XLRP) pattern. Eleven loci have been described for adRP on chromosomes 1q, 3q, 6p, 7p, 7q, 8cen, 11q, 14q, 17p, 17q and 19q, and the respective genes have been cloned except for the 17q locus.

We have recruited a four generation family with adRP after full clinical examination and we have genotyped 26 of its members (of which 9 are affected) with ~200 fluorescently labeled microsatellite markers. After analysis excluded linkage to the eleven known adRP loci, a genome-wide search was undertaken and twelve chromosomes were excluded in their entirety. Additional family members are also being recruited in order to strengthen these results.

In an effort to map novel disease-causing adRP genes we provide evidence for a novel disease locus for adRP. This is supportive of previously published data indicating the existence of additional adRP loci in the human genome. Once linkage is established, candidate genes from the corresponding genetic interval will be characterised and screened for mutations in affected members of the adRP family.

**A two-stage genome-wide scan identifies a new susceptibility locus for autosomal dominant keratoconus in a large Caucasian pedigree.** *Y. Tang<sup>1</sup>, K.D. Taylor<sup>1, 2</sup>, X. Li<sup>1</sup>, Y. Picornell<sup>1</sup>, Y.S. Rabinowitz<sup>1, 2</sup>, H. Yang<sup>1</sup>, Genetic Epidemiology.* 1) Dept Medical Genetics, Cedars-Sinai Medical Ctr, Los Angeles, CA 90048; 2) Dept Pediatrics, Cedars-Sinai Medical Ctr, Los Angeles, CA 90048.

Keratoconus (KC) is a corneal dystrophy with an incidence of 1 in 2,000. It is a major cause of visual disability worldwide and a leading cause for cornea transplantation in Western developed countries. The etiology of KC is unknown, but heterogeneous genetic determination in the pathogenesis of KC is supported by familial aggregation, twin concordance, and linkage studies. We previously conducted a linkage study on candidate chromosome 21 because of keratoconus' known association with Down's syndrome and we observed a region of suggestive linkage (LOD = 2.5) near the marker D21S1240. To further investigate genetic contributions in the development of KC, we extended the linkage analysis to the whole genome. We studied a five-generation autosomal dominant pedigree, consisting of 39 members, 14 of whom were affected with keratoconus (diagnosed both clinically and with videokeratography). We performed linkage analysis with 343 microsatellite markers along the 22 autosomal chromosomes at ~10 cM density. For those regions with suggestive linkage, fine mapping at ~2 cM density was carried out. Model-based multipoint linkage analysis was performed using GeneHunter2. Under the dominant model with a penetrance of 0.8, evidence of suggestive linkage from the initial scan was observed at 83-115 cM region on chromosome 5 with a maximum lod score (LOD) of 3.17 near marker D5S644. Further fine mapping was performed by testing additional 8 microsatellite markers at 2-3 cM intervals in a 30 cM area, revealing a similar, but narrower, peak (99-112 cM) with maximum LOD 3.03 near marker D5S484. These results indicate a promising new locus for keratoconus at D5S484 in this pedigree. Because of the heterogeneous nature of keratoconus, this locus may be specific to familial dominant keratoconus, and may not play an important role in sporadic KC. Nevertheless, the identification of this locus may provide new insights into the pathogenesis of keratoconus.

**Age-related maculopathy: A third genome-wide scan with continued evidence of susceptibility loci within the 1q31 and 17q25 regions.** *D.E. Weeks<sup>1</sup>, Y.P. Conley<sup>1</sup>, H.-J. Tsai<sup>1</sup>, T.S. Mah<sup>1</sup>, S. Schmidt<sup>2</sup>, M.A. Pericak-Vance<sup>2</sup>, J.L. Haines<sup>3</sup>, E. Postel<sup>2</sup>, P.J. Rosenfeld<sup>4</sup>, T.O. Paul<sup>5</sup>, A.W. Eller<sup>1</sup>, L.S. Morse<sup>6</sup>, J.P. Dailey<sup>7</sup>, R.E. Ferrell<sup>1</sup>, M.B. Gorin<sup>1</sup>.* 1) Univ Pittsburgh, Pittsburgh, PA; 2) Duke Univ Med Ctr, Durham, NC; 3) Vanderbilt Univ Med Ctr, Nashville, TN; 4) Univ. of Miami, Bascom Palmer Eye Inst., Miami, FL; 5) Smith-Kettlewell Eye Research Institute, San Francisco, CA; 6) University of California Davis, Davis, CA; 7) Case Western Reserve, Cleveland, OH.

Age-related maculopathy (ARM) or age-related macular degeneration is one of the most common causes of visual impairment in the elderly within developed nations. In a combined analysis of two previous genome-wide scans that included 391 families containing up to 452 affected sib pairs, we found evidence of linkage in four regions, 1q31, 9p13, 10q26, and 17q25. We now have added a third set of families, and have carried out an integrated analysis incorporating 530 families and up to 736 affected sib pairs. Using three diagnostic models, we have conducted linkage analyses using parametric (heterogeneity lod scores [HLOD] under an autosomal dominant model) and non parametric ( $S_{all}$  statistic) methods. There is ongoing evidence of susceptibility loci within the 1q31 and 17q25 regions. Within the 1q31 region, we observed an HLOD of 2.72 under our least stringent diagnostic model, while the 17q25 region contained a maximal HLOD of 3.53 under our intermediate diagnostic model. We have evaluated our results with respect to those from several new independent genome-wide linkage studies, and also have completed ordered subset analyses using ApoE alleles, smoking history, and age-of-onset as stratifying covariates.

**Genetic study of the Bardet-Biedl syndrome in Tunisia.** *N. Smaoui*<sup>1,2</sup>, *S. Li*<sup>1</sup>, *M. Chaabouni*<sup>2</sup>, *R. Mrad*<sup>2</sup>, *H. Kallel*<sup>3</sup>, *F. Maazoul*<sup>2</sup>, *E. Nouiri*<sup>2</sup>, *H. Chaabouni*<sup>2</sup>, *J.F. Hejtmancik*<sup>1</sup>. 1) Dept OGVFB, National Eye Inst, NIH, Bethesda, MD; 2) Genetic Department, Faculty of Medicine, Tunis, Tunisia; 3) Pediatric Department, Ras Jebel hospital, Bizerte, Tunisia.

Bardet-Biedl syndrome (BBS) is a clinically and genetically heterogeneous syndrome characterized by obesity, polydactyly, mental retardation, and retinitis pigmentosa. It is caused by mutations at least seven known loci including 11q23 (BBS1), 16q21 (BBS2), 3p13-p12 (BBS3, gene unknown), 15q22.3-q23 (BBS4), 2q31 (BBS5, gene unknown), 20p12 (BBS6) and 4q27 (BBS7). We carried out a linkage study of 9 consanguineous Tunisian families with microsatellite markers flanking each of the 7 BBS loci. One family shows linkage to D4S402 near the BBS7 locus with a maximum lod score of 5.42 at  $\theta = 0$ . Affected individuals in three families show homozygosity for microsatellite markers, one family each to markers flanking the BBS1, BBS2 and BBS6 loci. Sequencing of the corresponding candidate gene coding sequences is in progress. In family 57009 all 7 known loci for BBS have been excluded and a genome wide scan is underway with this family to identify the putative new Bardet-Biedl locus.

Program Nr: 1825 from 2003 ASHG Annual Meeting

**Possible digenic inheritance for limb girdle muscular dystrophy.** *A. Starling, F. De Paula, R. Pavanello, M. Vainzof, M. Zatz.* Dept Biologia, Inst de Biociencias, Sao Paulo, Brazil.

Limb Girdle Muscular Dystrophy (LGMD) is a neuromuscular disorder characterized by a great genetic heterogeneity. At least 15 LGMD-causing genes have been mapped so far, 5 with autosomal dominant (LGMD1A-E) and 10 with autosomal recessive inheritance (LGMD2A-J). We have ascertained a family with 5 affected brothers presenting proximal muscle weakness but with a variable course and preferential muscle involvement. They have 5 normal sibs (3 brothers and 2 sisters). All affected patients have grossly elevated serum CK (50-100 fold). A muscle biopsy performed in two of them showed normal results for dystrophin and 7 other analyzed proteins (dysferlin, calpain, the four sarcoglycans and telethonin). Linkage analysis excluded all known autosomal dominant and recessive LGMD genes. Interestingly, all five affected brothers inherited the 2362AG>TCATCT CAPN3 mutation in one allele but linkage analysis confirmed that the second allele does not carry a mutation in the CAPN3 gene. In order to find out if the disease is caused by tri-allelic inheritance (with the CAPN3 mutation acting as a modifier gene) or di-allelic inheritance we are currently performing a genome scan. The analysis of 450 markers (ABI Prism Linkage Mapping Set Version 2) excluded recessive inheritance for a novel LGMD gene and therefore tri-allelic inheritance. We are currently looking for a common allele in the affected brothers that may interact with the CAPN-3 mutation causing the LGMD phenotype. If confirmed, this will be the first LGMD family showing a digenic mechanism of inheritance. Supported by FAPESP-CEPID, PRONEX, CNPq.

**Linkage analysis of LRP5 gene with bone mass density.** *E. Petrelli<sup>1</sup>, A. Sangalli<sup>1</sup>, G. Malerba<sup>1</sup>, L. Xumerle<sup>1</sup>, V. Braga<sup>2</sup>, S. Adami<sup>2</sup>, P.F. Pignatti<sup>1</sup>, M. Mottes<sup>1</sup>.* 1) Mother & Child, Biol & Gen, Univ. Verona, Verona, Verona, Italy; 2) Dept of Rheumatology, Valeggio S/M, University of Verona, Italy.

In recent years several studies have reported mutations in the Lipoprotein Receptor-related Protein 5 gene (LRP5) associated to either increased or decreased bone density conditions. We are performing a linkage analysis in a sample of Italian osteoporotic families to investigate the possible involvement of the LRP5 gene with bone density in osteoporosis. Families (119) were recruited through an osteoporotic/osteopenic proband for a total of 563 individuals genotyped. The individuals were characterised for bone density at spine and femoral sites and for lipid levels. The allele frequency of 8 intragenic SNPs (Q89R, V1119V, IVS17-30, V1330A, D1363D, N740N, IVS10+6, TSCO178416) was estimated on 118 family founders. The SNP TSCO178416 (from SNP Consortium Database) was found not to be polymorphic. Bone density has been treated both as quantitative and qualitative trait. Non parametric linkage analysis for markers V1119V and V1330A did not show suggestive results with bone density at any of the densitometric sites studied. These preliminary results suggest that the LRP5 gene is not a major genetic factor for bone mass loss in osteoporosis.

**A Genome Scan for Asthma and a Combined Analysis of Two Asthma Collections.** *N.J. White<sup>1</sup>, M. Chiano<sup>1</sup>, S. Pillai<sup>2</sup>, R. Lam<sup>2</sup>, S. Brewster<sup>1</sup>, M. Ehm<sup>2</sup>, D. Burns<sup>2</sup>, M. Wagner<sup>2</sup>, J.H. Riley<sup>1</sup>, T. GAIN Investigators<sup>3</sup>, T. Progenitor Investigators<sup>3</sup>.* 1) GlaxoSmithKline, Medicines Research Centre, Molecular Genetics, Gunnels Wood Rd, Stevenage, Hertfordshire, UK; 2) 5 Moore Drive, RTP, North Carolina, USA; 3) See Acknowledgements.

Asthma is one of the most frequent, chronic diseases with a prevalence of up to 10%. It is a common disease, with contributions from both genetic and environmental factors. The asthma genetics program at GSK was launched in 1998 with a study termed GAIN (Genetics of Asthma International Network). The aim of this study was to collect 1000 families with Asthma. In 1998 GSK obtained a second asthma collection from a company called Progenitor. This collection contained approximately 300 families from Minnesota and Denmark. We have carried out a genome scan on the GAIN collection and reported the data last year at ASHG. We have recently carried out a genome scan and performed linkage analysis on the Progenitor families and will present this. Finally we have carried out a combined analysis of the two datasets and will present this analysis also. The Progenitor genome scan analysis was of four asthma sub-phenotypes, physicians diagnosed asthma (PDA), atopic asthma, strict asthma and bronchial hyper-responsiveness (BHR). The analysis was carried out separately in each of the Danish and Minnesota populations and a combined analysis of them both. A total of 8 peaks with a LOD greater than 1.0 were observed in the combined Minnesota, Denmark analysis with 2 of these peaks with LODs greater than 2.0. The GAIN scan had a total of 12 peaks with a LOD score greater than 1.0 in one or more phenotypes and 2 with LODs greater than 2. When the collections were combined and the four phenotypes analysed we had 5 loci with a LOD greater than 2.0 and 11 loci with a LOD greater than 1.0. We will discuss the additive nature of some of these chromosomal loci and how we will use this to prioritise the next stage of our analysis.

**A Genome-wide Linkage Scan for Schizophrenia in Veterans Affairs Cooperative Study Families.** *A.D. Skol<sup>1</sup>, S.V. Faraone<sup>2</sup>, D.W. Tsuang<sup>3</sup>, S. Bingham<sup>4</sup>, K.A. Young<sup>4</sup>, S.L. Haverstock<sup>4</sup>, F. Mena<sup>4</sup>, A.S. Menon<sup>4</sup>, F. Sautter<sup>4</sup>, J.L. Vanden Eng<sup>1</sup>, T. Keith<sup>5</sup>, M. Boehnke<sup>1</sup>, M.T. Tsuang<sup>6,7</sup>, G.D. Schellenberg<sup>8</sup>.* 1) Dept. of Biostatistics, University of Michigan, Ann Arbor, MI; 2) Harvard Medical School Department of Psychiatry at the Massachusetts General Hospital, Boston, MA; 3) Veterans Affairs Puget Sound Health Care System, Mental Illness, Research, Education and Clinical Center, Seattle, Washington; 4) Veterans Affairs Medical Center; 5) Genome Therapeutics, Inc., Waltham, MA; 6) Harvard Institute of Psychiatric Genetics, Boston, MA; 7) Harvard Department of Psychiatry at the Massachusetts Mental Health Center, Boston, MA; 8) Veterans Affairs Puget Sound Health Care System, Geriatric Research, Education and Clinical Center, Seattle, WA.

Schizophrenia is a serious psychiatric disease that affects around 1% of the population and has a known genetic component. We have nearly completed a genome scan using 166 families recruited from seven Veterans Affairs Medical Centers. The sample consists of 392 subjects affected by either schizophrenia (91.8%) or schizoaffective disorder, depressed (8.2%). Half of the families are northern European-American and half are African-American, representing one of the largest African-American samples analyzed to date.

In our preliminary findings, chromosome 18 contains the most significant result with a maximum LOD score of 3.68 located on the p-terminus, a previously unreported region of interest. When considered in concert with current literature our data suggests a number of other interesting results. These include a LOD score of 1.34 close to chromosome 11q23.3-24 which was found to be significantly linked with schizophrenia by Gurling et al. (2001), suggestive confirmation of Brzustowicz et al. (1999) chromosome 13q32 finding, a linkage peak on chromosome 15 (LOD = 0.67) only 1cM from the -7 nicotinic receptor subunit gene which was previously found associated with P50 auditory evoked potential gating deficits (Freedman et al., 2001), and suggestive confirmation of association between the NOTCH4 gene and schizophrenia found by Wei and Hemmings (2000.) The results from all chromosomes will be presented.

**Mapping of an X linked mental retardation (MRX) gene to a 9 Mb interval at Xq12.11.** *M. Frydman*<sup>1,2</sup>, *R. Attia*<sup>1</sup>, *E. Levy-Nissenbaum*<sup>1</sup>, *H. Reznik-Wolf*<sup>d</sup>, *M. Berkenstadt*<sup>1</sup>, *N. Schlaff*<sup>d</sup>, *E. Pras*<sup>1,2</sup>. 1) Danek Gertner Institute of Human Genetics, Haim Sheba Medical Centre, Tel Hashomer 52621, Israel; 2) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

**Purpose:** Linkage mapping of an X linked mental retardation (XLMR) gene in a new 3 generation family. **Clinical details:** 4 male patients had mild mental retardation and some facial similarity. One patient had atypical sensory motor neuropathy, hypertension, hyperlipidemia, and diabetes. Another patient had gynecomastia, and two patients had short ring-finger nails. **Results:** Linkage studies confined the disease gene into a 9 Mb interval on Xq12.11, between marker DXS8107 and a polymorphic site on clone AL009047. Two point LOD score of 3.52 at theta=0 was obtained with polymorphic sites on clones AC004386 and AL137013. This localization sets the new locus apart from the more centromeric OPHN1 and the telomeric FAFL4 genes. Since the interval is tangential to MRX22, telomeric to MRX1, MRX17, MRX55 and centromeric to MRX40, it defines a new locus for XLMR, which is partially or completely crossed only by MRX4, 5, 8, 13, 20, 26, 31, 52, 65, 69 and MRX77. Sequencing of ATR-X, NLGN3, CDX, SNX12, GPR23, NAP12 and GJB1 genes did not reveal any significant changes. Non random X inactivation pattern was found in all carrier females. Synteny of the inactive allele of the androgen receptor (AR) gene and the disease-linked haplotype was observed in all the carrier females. We suggest to classify XLMR conditions with skewed X inactivation as a separate class of non-specific mental retardation (MRX), since they present a subtle but central syndromic feature. Such classification may allow further refinement of the MRX phenotype. Determination of the inactivation pattern may be important for genetic counseling in female carriers who are not informative, and facilitate gene mapping in families which are too small to be studied based on male phenotype alone.

**Transcriptional map of the dominant intermediate Charcot-Marie-Tooth neuropathy locus on chromosome 19p12-p13.2.** *M.L. Kennerson<sup>1,2</sup>, D. Zhu<sup>1</sup>, G. Walizada<sup>1</sup>, G.A. Nicholson<sup>1,2</sup>*. 1) Neurobiology Laboratory, ANZAC Research Institute, Concord, Australia; 2) Molecular Medicine Laboratory, Concord Hospital, Concord, Australia.

Charcot-Marie-Tooth (CMT) neuropathy is one of the most common hereditary disorders of the human peripheral nervous system. Dominant intermediate CMT (CMTDI1) is a form of CMT with intermediate median motor nerve conduction velocities. We previously reported linkage of CMTDI1 to a 16.8-cM interval on chromosome 19p12-p13.2 in a large Australian kindred (1). Extended haplotype analysis of additional family members and the report of a second family (2) linked to this locus enabled us to narrow the candidate region to a 6-cM interval flanked by D19S558 and D19S432 (3). This region corresponds to a 2.5-Mb physical distance and is amenable to positional cloning strategies. We have employed resources of the Human Genome Project (HGP) to construct a sequence ready contig of BAC clones that span the CMTDI1 critical region. Both bioinformatic tools and laboratory techniques have been employed to annotate the DNA sequence and generate a comprehensive transcript map. Expression profiles in neural tissues (brain, spinal cord and peripheral nerve) have identified potential candidate genes that are being screened by direct sequencing. 1.Kennerson et al. *Am. J. Hum. Genet* (2001) 69:883-888. 2.Speer et al. *Neurogenetics* (2002) 4:83-85. 3.Zhu et al. *Neurogenetics* (2003) Epub ahead of print.

**Mapping of the Infantile Bilateral Striatal Necrosis gene in the chromosomal region 19q13.32-q13.41. M.**

*Shohat*<sup>1,2</sup>, *R. Straussberg*<sup>2,3</sup>, *H. Ovadia*<sup>4</sup>, *A. Kaplan*<sup>4</sup>, *N. Magal*<sup>2,4</sup>, *Z. Shorer*<sup>5</sup>, *H. Shalev*<sup>6</sup>, *C.A. Walsh*<sup>7</sup>, *L. Basel-Vanagaite*<sup>1,3</sup>. 1) Dept Medical Genetics, Rabin Medical Ctr, Petah Tikva, Israel; 2) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv; 3) Neurogenetics Clinic and Department of Pediatrics, Schneider Childrens Medical Center of Israel, Petah Tikva; 4) Felsenstein Medical Research Center, Petah Tikva; 5) Department of Child Neurology, Soroka Medical Center, Ben Gurion University, Beersheba; 6) Pediatrics Department, Soroka Medical Center, Beersheba, Israel; 7) Division of Neurogenetics and Howard Hughes Medical Institute, Beth Israel Deaconess Medical Center, and Department of Neurology, Harvard Medical School, Boston, Massachusetts, USA.

Background: Infantile bilateral striatal necrosis (IBSN) encompasses several syndromes of bilateral symmetrical degeneration of the caudate nucleus, putamen, and globus pallidus. Autosomal recessive infantile bilateral striatal necrosis (IBSN) is characterized clinically by developmental arrest beginning at the age of 7 to 15 months, dysphagia, choreoathetosis, pendular nystagmus and optic atrophy and severe progressive atrophy of the basal ganglia on MRI. Objective: To map the gene causing autosomal recessive IBSN. Methods: A 10-cM genomewide linkage scan was initially performed on 5 affected and 5 unaffected individuals. The extended family was included in the analysis to narrow the candidate region. LOD score was calculated using the SUPERLINK program. Results: Linkage to the chromosomal region 19q13.32-13.41 was established ( $Z_{max} = 6.27$  at  $\theta = 0.02$  at locus D19S412). Recombination events and a common disease-bearing haplotype defined a critical region of 1.2 Mb between the loci D19S596 proximally and D19S867 distally. Conclusion: Autosomal recessive IBSN maps to the chromosomal region 19q13.32-13.41. The presence of a common haplotype in all the patients suggests that the disease is caused by a single mutation derived from a single ancestral founder in all the families.

**QTL mapping for hypercalciuria in a rat model of kidney stone disease.** *S.J. Scheinman<sup>1</sup>, S. Sen<sup>2</sup>, R. Reid<sup>1</sup>, R.V. Thakker<sup>3</sup>, D.A. Bushinsky<sup>4</sup>, R.R. Hoopes, Jr<sup>1</sup>*. 1) Department of Medicine, SUNY Upstate Medical University, Syracuse, NY; 2) Department of Epidemiology and Biostatistics, University of California at San Francisco; 3) MRC Molecular Genetics Unit, University of Oxford, UK; 4) Department of Medicine, University of Rochester, Rochester NY.

The genetic hypercalciuric stone-forming (GHS) rat model was developed by selective breeding for hypercalciuria from Sprague-Dawley progenitors. Calcium excretion progressively increased for the first 30 generations, consistent with multiple loci contributing to hypercalciuria; the strain is now in its 64th generation of inbreeding. The GHS rat has been extensively characterized. The complex physiology of hypercalciuria in these rats involves increased intestinal calcium absorption, enhanced bone resorption, and impaired renal calcium reabsorption. The vitamin D receptor is overexpressed in these rats. Thus, the GHS rat model reproduces the human phenotype of idiopathic hypercalciuria, which is the most common risk factor for kidney stones. We studied an F2 generation of 234 rats bred from GHS females and normocalciuric WKY males. Ca excretion is 6- to 10-fold higher in the GHS than in the WKY progenitors. We performed quantitative trait locus (QTL) analysis on Ca excretion using the F2 rats and Pseudomarker QTL mapping methods designed to detect multiple, possibly linked or interacting, loci. After adjusting for the additive effects of sex and cross batch, we found significant evidence for five QTL, two of them linked. The loci were on chromosome 3 (2 peaks with LOD 4.92 and 3.50), 5 (LOD 4.08), 1 (LOD 3.75) and 14 (LOD 3.57). Further analysis indicated a sex effect for the QTL on chromosomes 5 and 14 ( $p < 0.01$ ). For the chromosome 5 locus the GHS allele is recessive for males, but dominant for females. We have bred N10F1 congenic rats preserving the GHS alleles for the chromosome 1 QTL on a WKY background, and these congenics are now available for study. More refined mapping using congenic strains will be needed to identify the genes at these loci contributing to hypercalciuria in rats and, potentially, in humans.

**DBH as a model of haplotype block utility in linkage disequilibrium mapping.** *C.P. Zabetian<sup>1</sup>, C.W. Wilkinson<sup>2, 3</sup>, A. Samii<sup>1</sup>, G.D. Schellenberg<sup>1, 2</sup>.* 1) Dept of Neurology; 2) GRECC; 3) Dept of Psychiatry; Univ of Washington and VAPSHCS, Seattle, WA.

Although recent findings suggest that much of the human genome is organized in blocks of low haplotype diversity, the utility of such blocks in identifying genes influencing common traits is not yet known and must ultimately be tested empirically using real data. One approach is to fine-map genes already known to affect common traits and determine if a prior knowledge of LD structure might have facilitated gene identification using marker densities appropriate for large scale studies. The DBH gene is well suited as a simple model for this purpose as it contains a putative functional single nucleotide polymorphism (SNP; -1021C>T) in the 5' upstream region that accounts for half of the total variance in plasma DBH activity levels in European-Americans (EAs). A recent study of 12 SNPs at DBH (Zabetian et al., *Am. J. Hum. Genet.* 2003; 72:1389-1400) identified a single 10 kb haplotype block that contained -1021C>T and found that only one of six markers located outside of this block was highly associated with phenotype. Here we present data from a map of 30 SNPs in a new group of EA subjects that replicate and extend these findings as follows: 1) Additional markers typed within the previously defined block-boundaries all conformed to block structure, and all intra-block markers were strongly associated with plasma DBH activity. One such marker, IVS5+192C>T (TaqI site), is of special interest because it is also associated with ADHD. 2) Most markers located outside the block containing -1021C>T showed little or no association to phenotype. 3) The remainder of the DBH gene contained several short haplotype blocks of 3 kb or less in length. Thus, if one had attempted to map quantitative trait loci for plasma DBH activity on a genomewide basis without prior knowledge of candidate regions and not included (by chance) markers within the block containing -1021C>T, the DBH locus might have been missed entirely. These results provide a direct example of the potential value of constructing a haplotype map of the human genome prior to embarking on large-scale association studies.

**Haplotype architecture of the norepinephrine transporter gene [*SLC6A2*] in four populations.** *I. Belfer*<sup>1</sup>, *G. Phillips*<sup>1</sup>, *J. Taubman*<sup>2</sup>, *M.B. Max*<sup>1</sup>, *D. Goldman*<sup>2</sup>. 1) Pain and Neurosensory Mechanisms Branch, NIDCR, NIH, Rockville, MD; 2) Laboratory of Neurogenetics, NIAAA, NIH, Bethesda, MD.

The norepinephrine transporter (NET) is a transmembrane protein responsible for norepinephrine reuptake. The *SLC6A2* missense substitution Ala457Pro alters transporter function in vitro. A synonymous substitution, G1247C has been used in a series of association studies to norepinephrine-related phenotypes including hypertension and mood disorders. However, these studies have in general been negative or equivocal. A haplotype approach combining missense polymorphisms with loci chosen for haplotype informativeness offers the potential for detection of unknown alleles of moderate abundance and effect. To elucidate *SLC6A2* haplotype block structure and SNP panel informativeness, a panel of 27 SNP markers including G1247C and Ala457Pro was selected across the 46 KB *SLC6A2* region and genotyped by the 5 nuclease method in 96 unrelated individuals from each of four ethnic populations: Finnish Caucasians, U. S. Caucasians, Plains Indians and African Americans. Genotyping error rate was directly determined and was <0.005. Genotype completion rate was 94%. Within *SLC6A2*, three conserved blocks [I: 13.6 Kb, II: 12.5 Kb, III: 25 Kb] showed evidence for little historical recombination except for some disruption of LD within Block II and shortening of Block III in African Americans. Within blocks, pairwise LD [D] was generally >0.9 for all locus pairs. For the three haplotype blocks, the numbers of SNPs were: U.S. Caucasians, Finns, and Plains Indians: 8, 8, and 9, and African Americans: 8, 8, and 8. The number of common [>0.05] haplotypes in these groups were: block I - 4, 5, 3, 6; block II - 5, 5, 4, 5, and block III - 4, 5, 5, 6, for the four populations as just listed. The 7 SNPs in block II without G1247C, the marker most extensively used in linkage studies, capture 99% of the information content of Block II when G1247 is included. The *SLC6A2* haplotype map and marker panel is a surrogate for unknown, but moderately abundant and functionally effective, *SLC6A2* alleles.

Program Nr: 1835 from 2003 ASHG Annual Meeting

**SNP Genotyping Production for the WICGR Hap Map Project.** *J.M. Moore<sup>1</sup>, B. Blumenstiel<sup>1</sup>, R. Chanoux<sup>1</sup>, M. Defelice<sup>1</sup>, M. Faggart<sup>1</sup>, M. Goyette<sup>1</sup>, A. Lochner<sup>1</sup>, H. Nguyen<sup>1</sup>, E. Stahl<sup>1</sup>, E. Winchester<sup>1</sup>, L. Ziaugra<sup>1</sup>, D. Altshuler<sup>1,2</sup>, S.B. Gabriel<sup>1</sup>.* 1) Ctr Genome Res, Whitehead Inst, Cambridge, MA; 2) Departments of Genetics and Medicine, Harvard Medical School; Department of Molecular Biology and Diabetes Unit, Massachusetts General Hospital, Boston, MA.

Our goal as part of the International Haplotype Map Consortium is to genotype a high density of markers across four chromosomal regions: 4q, 7q, 18p, and Y. We are currently in production level genotyping on the Sequenom MassArray platform. At the outset of the project, we scaled the SNP genotyping process over five-fold, enabling us to genotype >1,100 SNPs per day (per panel of 96 samples). The success rate of assay design using the Sequenom platform is >80% and the conversion rate to successful genotyping assays is 84% for the first 17,000 SNPs attempted. To select SNPs for production genotyping, we use a strategy which calls for genotyping "double-hit" SNPs preferentially to avoid genotyping of false positive from SNP discovery and to increase the yield of common SNPs. We find that 89% of candidate SNPs are validated in the CEPH DNA panel (120 chromosomes) and 72% of SNPs have a minor allele frequency > 10%. Genotyping accuracy is critical in the analysis that will generate the ultimate product of the Hap Map. Therefore, we continually monitor accuracy of production genotyping by checking for consistent segregation within families and concordance of duplicate genotypes. Overall our accuracy rate is approximately 99.8%.

**Microtape: an alternative to microtiter plates for high throughput genotyping and other applications.** A. Yu, T. Rusch, W. Dickinson, K. Fieweger, J. Chudyk, D. David, J. Weber. Center for Medical Genetics, Marshfield Medical Research Foundation, Marshfield, WI.

Microtiter plates are used today in nearly all genetics labs for genotyping, sequencing and other reactions. Microtape is a series of arrays of wells on a continuous thin strip of plastic. Microtape is cheaper to manufacture, easier to design, and more readily processed by automated instruments than individual microtiter plates. Two series of indexing holes that run along either edge of the microtape allow the tape to be automatically fed through various instruments. Many different well shapes and sizes can be created. Array geometries are also easily modified. Currently we are using 384-well arrays with the same geometry as 384-well plates and with brim well volume of about 1 l. We have developed a series of instruments to handle the tape and to carry out an allele-specific PCR genotyping assay. A 384-tip positive displacement pipetting instrument was built to transfer DNA (or PCR primers) from a reservoir plate to the arrays. Typically, the DNA or primers are dried at the bottoms of the wells. A solenoid micro-valve aspirating and jetting unit was developed for dispensing a common reaction mix into the wells. This instrument will dispense volumes ranging from about 25-2000 nl into each of 384 wells in about 1 min. Arrays are sealed using another custom built device with two closely spaced rollers, one of which is heated. After PCR within a waterbath-based thermal cycler, the arrays are scanned using an epi-fluorescence detection unit designed to read Fam and Joe fluorescent dyes. The resulting images are processed using software written in-house. In calendar year 2003 we will generate about 1 million diallelic polymorphism genotypes using the microtape. Current reaction volumes are 600 nl and dropping. At these low reaction volumes, reagent costs per genotype are only about 2. Recent genotyping completeness values in the microtape have ranged from 94%-98%, with error rates from 0.06%-0.35%. Microtape also can be applied to amplification of multiallelic polymorphisms and to other reactions.

**Large Scale SNP Genotyping with Molecular Inversion Probe (MIP) Technology.** *T. Willis<sup>1</sup>, P. Hardenbol<sup>1</sup>, O. Iartchouk<sup>1</sup>, C. Bruckner<sup>1</sup>, M. Moorhead<sup>1</sup>, E. Namsaraev<sup>1</sup>, G. Karlin-Neumann<sup>1</sup>, M. Jain<sup>1</sup>, F. Yu<sup>2</sup>, X. Lu<sup>2</sup>, S. Pasternak<sup>2</sup>, S. Ghose<sup>2</sup>, A. Perez<sup>2</sup>, D. Steffen<sup>2</sup>, S. Leal<sup>2</sup>, G. Scott<sup>2</sup>, J. Belmont<sup>2</sup>, R. Gibbs<sup>2</sup>.* 1) ParAllele BioScience, So San Francisco, CA; 2) Baylor College of Medicine, Human Genome Sequencing Center, One Baylor Plaza, Houston, Texas.

The challenge of inexpensively generating hundreds of thousands of genotypes is being met at the BCM-HGSC by the use of Molecular Inversion Probe, (MIP) technology as developed at ParAllele BioScience. MIPs are padlock probes that hybridize directly to genomic DNA and undergo a unimolecular rearrangement, or inversion, through the action of several enzymes. Inversion transforms a probe incapable of amplification into one that can - dependent on the genetic information of the SNP target. After inversion, probes are amplified by PCR using common primers and analyzed via universal sequence tag DNA microarrays. Several selective enzymatic steps in the process allow multiplexing to over 10,000 probes per assay and ensure highly accurate genotypes (99.8%). This technology has been successfully transferred to the BCM-HGSC, and is working robustly and routinely in a high throughput, production scale environment. The BCM-HGSC is responsible for genotyping, and ultimately developing a high quality Haplotype Map for Chromosome 12. By mid June 2003, the MIP technology had already yielded in excess of one million genotypes on 30 CEPH trios, with an impressive 90.49% assay success rate, a 96.3% genotype call rate, and an 80% conversion rate (randomly chosen unique SNPs in dbSNP). The average repeatability and Mendelian consistency were 99.77% and 99.69% respectively. Given that the size of Chromosome 12 is about 133 Mb an average density of one SNP per 12.6 kb has already been achieved. Early haplotype results of chromosome 12 indicate that haplotype blocks, as determined by linkage disequilibrium of at least 4 SNPs per block, currently cover 22% of the chromosome with a median block size of 40kb at the current average density of 1 marker per 8kb. Taken as a whole, early results from the high throughput use of this MIP genotyping technology are highly encouraging and demonstrate the strong utility of this multiplexed genotyping assay.

**Association of calsequestrin 1 on 1q21 with type 2 diabetes mellitus (T2DM) in the Old Order Amish.** *M. Fu<sup>1</sup>, C. Damcott<sup>1</sup>, M. Sabra<sup>1</sup>, S. Ott<sup>1</sup>, J. Lee<sup>1</sup>, T.I. Pollin<sup>1</sup>, M. Garant<sup>1</sup>, J. O'Connell<sup>1</sup>, B.D. Mitchell<sup>1</sup>, A.R. Shuldiner<sup>1, 2</sup>.* 1) Dept Med, Div Endocrinology, Univ Maryland, Baltimore, MD; 2) Geriatrics Research and Education Clinical Center, Baltimore Veterans Administration Medical Center, Baltimore, MD.

We have previously detected linkage of T2DM to chromosome 1q21-q24, a region to which linkage has been demonstrated in several other populations. Calsequestrin 1 (CASQ1), a key storage protein of luminal Ca(2+) in the sarcoplasmic reticulum of muscle, is an attractive candidate gene in this region. We hypothesized that variation in CASQ1 may affect insulin action on glucose uptake and glycogen synthesis in skeletal muscle, thus increasing susceptibility to type 2 diabetes. We screened the 11 exons, -the surrounding intronic sequences, and the proximal regulatory region of CASQ1 in 20 Amish samples. We detected four novel SNPs in CASQ, including a C593T silent mutation in exon 3 (E3; Genbank accession number: NM\_001231), AG at 1470 bp upstream of the start codon (SPF1), del C at 1456 bp upstream of the start codon (SPF2), and del C at 1366 bp upstream of the start codon (SPF3). These SNPs as well as 12 other CASQ1 SNPs from dbSNP were genotyped in Amish subjects with T2DM (n = 150), impaired glucose homeostasis (IGH)(n = 148), and normal glucose tolerant (NGT) controls (n = 385), and association analyses were performed. 5 of the 16 SNPs genotyped showed significant association with T2DM, including rs3747623 in intron 2 (P=0.0257), E3 (P=0.0261), rs227503 in intron 4 (P= 0.0015), rs822450 in intron 7 (P=0.0481) and rs688015 (P=0.0240). Furthermore, all of five of these SNPs were genotyped in an expanded set of 1200 Amish subjects and showed significant association with post challenge glucose levels (736 Amish subjects have post-challenge glucose traits available). These SNPs define at least three distinct haplotype blocks, suggesting allelic heterogeneity. We conclude that variation in CASQ1 is associated with T2DM. Further studies of the role of CASQ1 in glucose homeostasis and the functional consequences of common variations in CASQ1 are warranted.

Program Nr: 1839 from 2003 ASHG Annual Meeting

**Bimodal distribution of sensitivity to the savory taste of monosodium glutamate (MSG) and polymorphisms of known glutamate taste receptors.** *P.A.S. Breslin, D.R. Reed, R.S.J. Keast, C.D. Tharp, S. Lui, O.M. Ohmed.* Monell Chemical Senses Ctr, 3500 Market St., Philadelphia, PA. 19104.

Monosodium glutamate (MSG), when placed in the mouth, conveys a unique taste quality, often labeled savory, brothy, or 'umami'. This quality is thought to underlie the protein and amino acid tastes, which convey a highly preferred flavor in many foods, e.g., soups. But not all of the population at large can clearly taste the savory component of MSG. A small subset of the population (~6%) is insensitive to this unique taste quality. Our subjects were screened for their ability to distinguish the tastes of 29 mM NaCl vs 29 mM MSG in a forced-choice triangle test. The few subjects who could not discriminate these stimuli also demonstrated an absolute insensitivity to glutamate on a concentration-intensity scaling task. Furthermore, the synergy of savory taste that occurs for most subjects when MSG is mixed with 5' ribonucleotides, such as GMP or IMP, is lacking in selected individuals, especially with regard to IMP. This specific lack of taste synergy can occur despite normal sensitivity to MSG savoriness. Therefore, there are semi-independent mechanisms underlying sensitivity to MSG and its interactions with IMP and GMP. At present, there are two putative savory taste metabotropic receptors identified: human taste GRM4 [6p21.3] and the receptor dimer human TAS1R1-TAS1R3 [1p36.23; 1p36.33]. Taste GRM4 (an orally located splice variant of the CNS GRM4, the group III inhibitory glutamate receptor) was sequenced and several identified exonic polymorphisms were not correlated with savory taster status. Similarly, the common polymorphism in TAS1R3 (A5T) was also not correlated with savory taster status. Less common polymorphisms, however, as well as a splice variant are being examined in our laboratory. TAS1R1 remains a likely location for a polymorphism that could determine MSG taster status. Four splice variants of TAS1R1 also hold promise as possible determinants of the MSG phenotypes under investigation. These studies have the potential to definitively determine the receptors responsible for this quality of taste in humans. This research was funded by DC02995. breslin@monell.org.

**Linkage and Association of polymorphisms in The SMOC2 Gene with Pulmonary Function: The Framingham Heart Study.** *S. Karamohamed*<sup>1</sup>, *J.B. Wilk*<sup>1</sup>, *C.M. Shoemaker*<sup>1</sup>, *J. Liu*<sup>1</sup>, *R.H. Myers*<sup>1</sup>, *D.J. Gottlieb*<sup>2</sup>. 1) Dept Neurology, Boston Univ Sch Medicine, Boston, MA; 2) The Pulmonary Center, R304, Boston University School of Medicine, Boston, MA.

Chronic obstructive respiratory disease is a significant cause of mortality and morbidity in the American population. To identify genes that affect lung function, we followed up linkage results on chromosome 6 in 191 families from the Framingham Heart Study using FEV1 and FVC as quantitative traits. We found linkage to FEV1 (LOD 5.0) and FVC (LOD 1.76) at 184.5 cM. To further refine the locus influencing FEV1 and FVC, we typed 25 single nucleotide polymorphisms (SNPs) covering a 1.5 Mb region in 1811 unrelated individuals. The SNPs data were analyzed using multiple linear regression models incorporating gender, age, body mass index (kg/m<sup>2</sup>), height, and smoking history as covariates. SNP 11 was associated in non-smokers with measures of FEV1 (p<0.001) and FVC (p<0.001). SNP 16, about 12 Kb 5'-distal to SNP 11, also showed similar but less significant association in non-smokers with FEV1 (p<0.08) and FVC (p<0.05) levels. SNPs 11 and 16 lie in two different haplotype blocks. Haplotype analysis using Haplo.score with SNP 11 and 16 revealed an association with FEV1 (p<0.009) and FVC (p<0.013) for non-smokers. The GT haplotype (76.6%) was associated with reduction of about 100 mL for FEV1 (p<0.004) and about 135 mL for FVC (p<0.002). Adjustment for SNP 11 in linkage models in the 191 families reduced the LOD score by one LOD unit. SNPs 11 and 16 lie within the SPARC related modular calcium binding 2 (SMOC2) gene. Further studies of SMOC2 are underway to define its role in lung and airway development.

**Quantitative trait locus analysis reveals two intragenic sites that influence O<sup>6</sup>-alkylguanine-DNA**

**alkyltransferase activity in peripheral blood mononuclear cells.** *M.F. Santibanez Korej<sup>d</sup>, J. Heighway<sup>2</sup>, G. McGown<sup>3</sup>, M.R. Thorncroft<sup>3</sup>, K.L. Harrison<sup>4</sup>, A.J. Watson<sup>3</sup>, P.V. Barber<sup>5</sup>, P.N. O'Donnell<sup>5</sup>, S.J. Lewis<sup>4</sup>, A.C. Povey<sup>4</sup>, G.P. Margison<sup>3</sup>.* 1) Institute of Human Genetics, University of Newcastle, Newcastle upon Tyne, United Kingdom; 2) Roy Castle International Centre for Lung Cancer Research, Liverpool, United Kingdom; 3) Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Manchester, United Kingdom; 4) Centre for Occupational and Environmental Health, University of Manchester, Manchester, United Kingdom; 5) North West Lung Cancer Centre, Wythenshawe Hospital, Manchester, United Kingdom.

The DNA repair protein, O<sup>6</sup>-alkylguanine-DNA alkyltransferase (MGMT) is a major determinant of resistance to the mutagenic and toxic effects of certain classes of alkylating agents. The levels of expression of this protein are therefore of interest in relation to the prevention and treatment of cancer in man. They vary widely between individuals, and the basis of this variation is not understood. Here we demonstrate that in peripheral blood mononuclear cells (PBMC), the two MGMT alleles are frequently expressed at different levels, suggesting that there is a genetic component to inter-individual variation of MGMT levels and that at least some of the basis of this variation maps close to or within the MGMT locus. Next, we show by quantitative trait locus analysis using single nucleotide polymorphisms spanning the locus that there are at least two sites influencing interindividual variation in MGMT activity in PBMC. Characterisation of the relationship between alleles at these sites and expression levels allows to infer protein activity in specific individuals. Initial results from a case-control series suggest a link between MGMT activity and lung cancer risk.

***TNF-* is associated with joint pain in Hereditary Haemochromatosis.** *E.J. Walker*<sup>1</sup>, *S.R. Wilson*<sup>2</sup>, *H.R. Rodgers*<sup>3</sup>, *M.L. Bassett*<sup>3</sup>, *J.A. Cavanaugh*<sup>3</sup>. 1) Molecular Biosciences, JCSMR, ANU, ACT, Australia; 2) Centre for Mathematics and its Applications, ANU, ACT, Australia; 3) The Canberra Hospital, ACT, Australia.

**INTRODUCTION:** Hereditary haemochromatosis (HH) is an autosomal recessive disorder leading to excess iron storage<sup>1</sup>. Symptoms include joint pain, lethargy and loss of libido. The disorder results primarily from mutations in the *HFE* gene<sup>2-3</sup>. A previous study has suggested the -308 *TNF-* promoter polymorphism may have a role in the severity of clinical presentation<sup>4</sup>. **METHODS:** A total of 117 control DNA samples and 80 HH probands (defined by clinical presentation and genotype) were genotyped for the -308 *TNF-* polymorphism by PCR-RFLP<sup>5</sup>. HH Patients reported the presence or absence of joint pain via survey. Allele frequencies were compared by counting (<sup>2</sup> Test or Fisher's Exact Test as appropriate). Serum ferritin results were compared by the Mann-Whitney test. **RESULTS:** We compare allele frequencies for controls to HH patients (G allele 0.77 vs 0.86; p0.025), and HH patients with and without joint pain (G allele 0.75 vs 0.88; p0.05). We also compare mean serum ferritin levels (g/L) in HH patients (GG-1572.8; GA-1586.7; AA-720) according to *TNF-* genotype (not significant). **CONCLUSION:** The -308 *TNF-* polymorphism is associated with level of *TNF-* expression<sup>6</sup>. We found the allele frequency at this SNP to be significantly different in controls vs. HH probands. Within the HH group we also observed a significant difference in allele frequency between patients who experienced joint pain and those who did not. This difference was not a result of differences in serum ferritin (data not shown). No other symptom showed a significant correlation with the polymorphism. It has previously been suggested that lower serum ferritin levels in HH may correlate with this polymorphism<sup>4</sup>, however we failed to demonstrate a statistically significant difference in serum ferritin levels related to the -308 *TNF-* genotype. **REFERENCES:** 1. Bacon et al 1999 Gastroent 116:193; 2. Feder et al 1996 Nat Genet 13:399; 3. Mura et al 1999 Blood 93:2502; 4. Fargion et al 2001 Blood 97:3707, 5. Van Heel et al 2002 Hum Mol Genet 11:1281; 6. Gonzalez et al 2003 Am J Gastro 98:1101.

**Susceptibility and modifier genes in familial amyloid polyneuropathy (FAP) V30M.** *M.L. Soares*<sup>1,2</sup>, *T. Coelho*<sup>3</sup>, *A. Sousa*<sup>4</sup>, *S. Batalov*<sup>5</sup>, *I. Conceição*<sup>6</sup>, *M.L. Sales-Luís*<sup>6</sup>, *M.D. Ritchie*<sup>7</sup>, *S.M. Williams*<sup>7</sup>, *M.J. Saraiva*<sup>2</sup>, *J.N. Buxbaum*<sup>1</sup>. 1) Division of Rheumatology Research, MEM, The Scripps Research Institute, La Jolla, CA; 2) Amyloid Unit, IBMC, Porto, Portugal; 3) Centro de Estudos de Paramiloidose, Porto; 4) UnIGENe, IBMC, Porto; 5) Computational Biology Dept, Novartis Research Foundation, San Diego, CA; 6) Dept of Neurology, Hospital de Santa Maria, Lisbon, Portugal; 7) Program in Human Genetics and Dept of Molecular Physiology, Vanderbilt Univ Medical School, Nashville, TN.

FAP is an autosomal dominant disorder associated with mutations in the transthyretin (TTR) gene. Variability in penetrance and age of onset among carriers of the same mutation suggests that non-allelic genetic variations contribute to disease phenotype. To identify possible genetic modifiers in FAP we examined *HSPG2* (perlecan), *APCS* (serum amyloid P-component), *APOE* (apolipoprotein E), *SAAI*, *SAA2* (serum amyloid A) and *RBP4* (retinol binding protein) genes for associations with disease onset and/or susceptibility in subsets of mutant gene carriers representing classical- (under 40) and late- (over 50) onset cases. These genes encode non-fibrillar components of TTR amyloid deposits, or interact with TTR. Polymorphisms at these loci were analyzed for single- and multiple-locus effects in a Portuguese sample of V30M carriers and unrelated controls. Single-locus analyses revealed that polymorphisms in *APCS* and haplotypes assembled for *APCS* and *RBP4* were significantly more common in V30M carriers than in the control subjects. When we tested for multiple-locus associations (linkage disequilibrium), there were no significant deviations from random in the controls (6 expected by chance alone). However we identified 25 significant combinations among the late-onset patients, while only 4 were seen in the classical-onset subset. Multifactor dimensionality reduction (MDR) indicated that the best genetic model for classical onset vs. controls resided predominantly in the *APCS* gene, while for the late onset cases *APCS* and *RBP4* were involved. The results indicate that genetic interactions among multiple loci, rather than single-locus effects, are likely to govern the age of onset in FAP V30M.

**Association of RGS4 polymorphisms with schizophrenia: interactions with gender.** *M.E. Talkowski<sup>1</sup>, K.V. Chowdari<sup>1</sup>, J.A. Wood<sup>1</sup>, J.W. Ceilley<sup>1</sup>, R.E. Ferrell<sup>1</sup>, K. Mirnics<sup>1</sup>, D.A. Lewis<sup>1</sup>, P. Levitt<sup>1</sup>, B. Devlin<sup>1</sup>, N. Williams<sup>2</sup>, M.J. Owen<sup>2</sup>, G. Kirov<sup>2</sup>, M.C. O'Donovan<sup>2</sup>, V.L. Nimgaonkar<sup>1</sup>.* 1) Psychiatry, Human Genetics, Neuroscience, and Neurobiology, University of Pittsburgh, Pittsburgh, PA; 2) Psychological Medicine, University of Wales College of Medicine, Cardiff, UK.

Gene expression and mapping studies have recently implicated the regulator of G-protein signaling 4 (RGS4) in the pathogenesis of schizophrenia (SCZ). We previously reported on evidence supporting association and linkage of a haplotype encompassing four SNPs at RGS4 (SNPs 1, 4, 7, 18) (Chowdari et al, 2002). Here, we report on interactions between this locus and gender in four independently ascertained case-parent samples. Exploratory analyses of clinical sub-groups in a sample from Pittsburgh revealed significant allele frequency differences between male and female patients for all four SNPs composing the associated haplotype (n=91). Increased homozygosity for the G allele was detected among female patients for each of these four SNPs. Significant gender differences were not evident for RGS4 SNPs not associated with SCZ, nor were they observed for the RGS4 SNPs among population-based controls, parents of the cases, or patients with Bipolar I disorder. The gender effects thus appeared to be specific, motivating analysis of samples ascertained by other groups. We found significant increases in homozygosity of SNP1 in female patients of a SCZ sample ascertained by the NIMH Collaborative Genetics Initiative (n=36). A similar trend was obtained in a sample of Indian patients (n=239), but such trends were not evident in a sample from Cardiff, UK (n=677). The results of analyses from UPITT, NIMH, and Indian samples suggest an interaction of gender with RGS4 in the pathogenesis of SCZ. However, analysis of samples obtained from Cardiff may imply that these gender related effects were chance observations. Alternatively, they may reflect a primary association with a variable correlated with gender. Our preliminary analyses suggest that the increased homozygosity is restricted to women with an early age at onset. Thus, further investigations are being conducted to probe other variables associated with disease severity in SCZ.

**Neurogenin 3 contributes to the linkage to T2DM on chromosome 10q23 in Italians.** *C. Gragnoli*<sup>1,2,4</sup>, *J.F. Brittman*<sup>1</sup>, *G. Menzinger von Preussenthal*<sup>4</sup>, *J.F. Habener*<sup>1,2,3</sup>. 1) Molecular Endocrinology, MGH, Boston, MA; 2) Med. Sch., Harvard Univer., Boston, MA; 3) HHMI, Boston, MA; 4) Univ. Tor Vergata di Roma, Italy.

Our goal is to identify the genes responsible for the linkage to type 2 diabetes mellitus (T2DM) on chromosome 10q23 in the Italian population. We genotyped chromosome 10 in 148 Italian late-onset T2DM sibs. Multipoint non-parametric linkage analysis (Merlin 2000-2002/decode map) shows a peak at d10s1213. Analysis of 105 affected sibs (conditional for d10s1213) show peaks at d10s556 and d10s1665. The D10s1665 marker is physically close to neurogenin 3 (*ngn3*), essential for pancreatic endocrine cell differentiation. We genotyped at d10s1665 129 late-onset sibs/22 early-onset T2DM families and obtained a 1.08 np score. (in only 22 early-onset T2DM families, np 1.64 score). We screened *ngn3* gene in 86 single T2DM patients/101 Italian controls and identified several variants: a new -152ntC/G, delCA44-45nt, Gly167Arg, Phe199Ser SNPs and two new promoter variations (-nt498GT/nt364CT). DelCA44-45/Phe199Ser variants, Gly167Arg/Phe199Ser, show significant LD (HPPlus software). The 152nt variation does not cosegregate with disease, and is not conserved in other species. Therefore, we suggest it is not causing T2DM. DelCA44-45, located at transcription factor binding sites, is not significantly associated with T2DM. Gly167Gly is conserved among species, allele A coding for 167Arg shows an association trend toward T2DM in 61 patients linked to d10s1665 (p-value=0.07/C.I.0.92-4.6) and allele G seems to protect from disease (p-value=0.07/C.I.0.22-1.09). A insC167 mutation present in one patient is shared by 2/3 diabetic family members and is absent from the controls. Phe199Phe is conserved among species, and CC/TC199 together with GA/AA167 show an association trend towards disease (p-value=0.12/C.I.0.83-4.28). Non-parametric linkage analysis within the *ngn3* gene variants in 6 early-onset T2DM families shows a np score=3.32/p-value=0.0005. Therefore *ngn3* variations in these 6 families are in linkage with T2DM. In our study GA/AA167 genotypes contribute with an OR=2.01 (C.I.0.93-4.35/p-value=0.07) to T2DM in our population.

**Localization of Novel Loci involved in Ocular and Cutaneous Melanoma.** *E. Gillanders<sup>1</sup>, S. Juo<sup>2</sup>, M. Jones<sup>1</sup>, C. Markey<sup>1</sup>, E. Holland<sup>4</sup>, N. Gruis<sup>6</sup>, B. Bressac-de Paillerets<sup>8</sup>, A. Goldstein<sup>5</sup>, M. Tucker<sup>5</sup>, G. Mann<sup>4</sup>, J. Bailey-Wilson<sup>2</sup>, J. Trent<sup>9</sup>.* 1) Cancer Genetics Branch/NHGRI/NIH, Bethesda, MD; 2) Inherited Disease Research Branch/NHGRI/NIH, Baltimore, MD; 3) Queensland Institute of Medical Research Unit, Brisbane, Australia; 4) Westmead Institute for Cancer Research, University of Sydney at Westmead Millennium Institute, Sydney, Australia; 5) Genetic Epidemiology Branch/NCI/NIH, Bethesda, MD; 6) Department of Dermatology and Center for Human and Clinical Genetics, LUMC, Leiden, The Netherlands; 7) Institut Curie, Paris, France; 8) Institut Gustave Rousy, Villejuif, France; 9) Translational Genomics Research Institute, Phoenix, AZ.

Over the past 20 years, the incidence of cutaneous malignant melanoma (CMM) has increased dramatically worldwide. A positive family history of the disease is among the most established risk factors for CMM; it is estimated that 10% of melanoma cases result from an inherited predisposition. Although mutations in two genes, CDKN2A and CDK4, have been shown to confer an increased risk of CMM, they account for only 20%-25% of families with multiple cases of CMM. Therefore, to localize additional loci involved in melanoma susceptibility, we have performed a genome-wide scan for linkage in 49 Australian pedigrees containing at least three CMM cases, in which CDKN2A and CDK4 involvement has been excluded. In order to minimize genetic heterogeneity we explored subset analyses based on a clinical presentation of both cutaneous and ocular melanoma. Follow up analyses of 2 regions with LOD scores greater than 2 will be presented.

**Association of an orexin 1 receptor 408Val variant with polydipsia-hyponatremia in schizophrenics. J.M.**

*Meerabux<sup>1</sup>, Y. Y Iwayama-Shigeno<sup>1</sup>, H. Ohba<sup>1</sup>, T. Toyota<sup>1,2</sup>, K. Yamada<sup>1</sup>, H. Shimizu<sup>3</sup>, K. Yoshitsugu<sup>1</sup>, K. Ohta<sup>4</sup>, T. Yoshikawa<sup>1</sup>.* 1) Molecular Psychiatry, Brain Science Institute, Riken, Saitama, Japan; 2) Department of Neuropsychiatry, Tokyo Medical and Dental University, Tokyo 113-8519, Japan; 3) Hokushin General Hospital, Nakano, Nagano 383-8505, Japan; 4) Onda-daini Hospital, Matsudo, Chiba 270-2251, Japan.

Polydipsia is a common and often life threatening complication in patients with chronic psychoses, particularly schizophrenia<sup>1</sup>. Research into the genetic etiology of polydipsia is limited and the pathogenesis of the disease remains poorly understood. One contributory factor is thought to be dopamine dysregulation caused by prolonged treatment with neuroleptics<sup>2</sup>. In this study, we put forward role for orexin (also known as hypocretin) signalling in the development of polydipsia. This is based on orexins physiological effect of promoting water intake in experimental animals<sup>3</sup> and the close anatomical and functional link between orexin neurons and the dopaminergic system<sup>4</sup>. We performed mutation screening on the genes for prepro-orexin and orexin receptors 1 (HCRTR1) and 2, and identified six polymorphisms. One mutation, a 408Ile-to-Val variant of HCRTR1 showed significant association with polydipsic-hyponatremic schizophrenics (P = 0.0138). The accumulation of this mutation was more pronounced in polydipsic compared to non-polydipsic schizophrenics (P = 0.0005; odds ratio = 1.83 [95% CI = 1.19 2.80]). These results suggest that individuals carrying the 408Val variant may show an increased risk for polydipsia-hyponatremia after chronic treatment with neuroleptics.

**Strong pattern of association with AD in a 210 kb region on chromosome 12.** *P. Nowotny<sup>1</sup>, Y. Li<sup>2</sup>, P. Holmans<sup>3</sup>, S. Smemo<sup>1</sup>, K. Tacey<sup>2</sup>, L. Doil<sup>2</sup>, K. Lau<sup>2</sup>, J. Catanese<sup>2</sup>, V. Garcia<sup>2</sup>, A. Myers<sup>4</sup>, V. Wavrant-DeVrieze<sup>4</sup>, S. Lovestone<sup>7</sup>, L. Thal<sup>5</sup>, L. Jones<sup>6</sup>, J. Williams<sup>6</sup>, M. Owen<sup>6</sup>, J. Hardy<sup>4</sup>, A. Goate<sup>1</sup>, A. Grupe<sup>2</sup>.* 1) Dept Psychiatry, Washington Univ Sch Medicine, St Louis, MO; 2) Celera Diagnostics, Alameda, CA, USA; 3) MRC Biostatistics Unit, Cambridge, UK; 4) NIA, Bethesda, MD, USA; 5) Dept of Neuroscience, UCSD, San Diego, CA, USA; 6) Dept of Psychological Medicine, Cardiff, UK; 7) Dept Neuroscience, Institute of Psychiatry, London, UK.

Several studies, including our own, have reported linkage to markers on chromosome 12 in late onset Alzheimer's disease (LOAD) families. The linkage signal comes predominantly from individuals with no APOE4 alleles. To follow up these results we have genotyped 473 SNPs in positional candidate genes on chromosome 12. The SNPs were initially tested in a case-control sample of 419 cases and 375 controls. Significant SNPs ( $p < 0.05$ ) were followed up in two other case-control sets (1428 samples total) and in the sample used for the linkage screen. The exploratory study revealed a strong pattern of association in a 210kb region in the whole set ( $p = 0.00002$ ) and the APOE4 negative subgroup ( $p = 0.0003$ ). Based on these initial results, 37 markers from this region were genotyped in the other case-control sets; results were summarized in a meta analysis of all samples. Pair wise inter marker LD patterns between cases and controls and across sample sets were very similar. In the meta analysis, 10 markers within the interval show significant association with  $p$ -values between 0.004 and 0.041 in the APOE4 negative subgroup. 7 SNPs, which define over 80% of the haplotype diversity in this region were genotyped in cases from the linkage sample and unrelated controls and a multilocus genotypic analysis was performed. Analysis of 6 SNPs simultaneously replicated the initial observation in the case control series ( $\chi^2 = 18.61(6df)$ ,  $p = 0.0049$ ). However the best-fitting model was achieved by using 4 loci ( $\chi^2 = 17.39(4df)$ ,  $p = 0.0016$ ). These results strongly suggest that an AD susceptibility allele is located within this region of chromosome 12.

**Functional SNPs in the lymphotoxin- gene that are associated with susceptibility to myocardial infarction.** K. Ozaki<sup>1</sup>, Y. Ohnishi<sup>1</sup>, A. Iida<sup>1</sup>, R. Yamada<sup>1</sup>, T. Tsunoda<sup>1</sup>, A. Sekine<sup>1</sup>, H. Sato<sup>2</sup>, H. Sato<sup>2</sup>, M. Hori<sup>2</sup>, Y. Nakamura<sup>1, 3</sup>, T. Tanaka<sup>1</sup>. 1) SNP Research Center, RIKEN, Tokyo, Japan; 2) Osaka University Graduate School of Medicine, Shita, Japan; 3) Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

By means of a large-scale, case control association study using 92,788 gene-based single nucleotide polymorphism (SNP) markers, we identified a candidate locus on chromosome 6p21 associated with susceptibility to myocardial infarction. Subsequent linkage-disequilibrium (LD) mapping and analyses of haplotype structure showed significant associations between myocardial infarction and a single 50 kb haplotype comprised of five SNPs in *LTA* (encoding lymphotoxin-), *NFKBIL1* (encoding nuclear factor of light polypeptide gene enhancer in B cells, inhibitor-like 1) and *BAT1* (encoding HLA-B associated transcript 1). Homozygosity with respect to each of the two SNPs in *LTA* was significantly associated with increased risk for myocardial infarction (odds ratio=1.78,  $\chi^2=21.6$ ,  $P=0.0000033$ ; 1,133 affected individuals versus 1,006 controls). *In vitro* functional analyses indicated that one SNP in the coding region of *LTA*, which changed an amino-acid residue from threonine to asparagine (Thr26Asn), effected a twofold increase in induction of several cell-adhesion molecules, including VCAM1, in vascular smooth-muscle cells of human coronary artery. Moreover, the SNP, intron 1 of *LTA*, enhanced the transcriptional level of *LTA*. These results indicate that variants in the *LTA* are risk factors for myocardial infarction and implicate *LTA* in the pathogenesis of the disorder.

**Association and linkage disequilibrium between synapsin 2 and complexin 2 gene and Korean schizophrenia.** *H.J. Lee<sup>1,2, 5</sup>, J.W. Kim<sup>2</sup>, M.K. Kim<sup>3</sup>, S.J. Kim<sup>2</sup>, S.Y. Jin<sup>1</sup>, M.S. Hong<sup>1,2</sup>, H.J. Park<sup>2</sup>, D.H. Shin<sup>4</sup>, J. Chung<sup>1,2</sup>, H. Shibata<sup>5</sup>, Y. Fukumaki<sup>5</sup>.* 1) Department of Pharmacology, College of Medicine, Kyung Hee University, Seoul, Republic of Korea; 2) Kohwang Medical Research Institute, College of Medicine, Kyung Hee University, Seoul, Republic of Korea; 3) Seoul National Mental Hospital, Seoul, Republic of Korea; 4) Department of Preventive School of Medicine, Medicine, Keimyung University, Degeu, Republic of Korea; 5) Division of Disease Genes Research Center for Genetic Information, Medical Institute for Bioregulation, Kyushu University, Fukuoka, Japan.

One of the pathophysiologic hypotheses of schizophrenia is aberrant functional connectivity. Recent studies reported that level of synapsin 2 (SYN2) and complexin 2 (CPLX2) mRNA in sections of brain from schizophrenia patients was decreased. In the current study, we analyzed several variants in both genes and made intermarker linkage disequilibrium (LD) maps. Correlation between  $D'$  and physical distance was observed between variants for less than 50kb at SYN2 and less than 10kb at CPLX2. Also, we performed association analysis of SYN2 and CPLX2 between 170 normal healthy controls and 158 schizophrenia patients. First, our results indicated general LD map in SYN2 and CPLX2. Second, there were several significant differences between controls and schizophrenia group in both SYN2 and CPLX2 by pairwise association analysis. ( $p < 0.05$ ) These results provide a potential value of constructing haplotype map and suggest that both SYN2 and CPLX2 polymorphisms may confer increased susceptibility to schizophrenia in Korean.

**Identification of a gene causing the limb malformation using a mouse model, Dominant hemimelia(Dh).** *S.K. Koo<sup>1</sup>, H.S. Jang<sup>1</sup>, J.Y. Heo<sup>1</sup>, S.C. Jung<sup>1</sup>, B. Oh<sup>2</sup>.* 1) Division of Genetic Diseases, Department of Biomedical Science, National Institute of Health, Korea, Seoul, South Korea; 2) Division of Epidemiology and Bioinformation, National Genome Research Institute, National Institute of Health, Korea, Seoul, South Korea.

To understand the pathogenesis of limb malformation, the polydactyly mouse, Dominant hemimelia (Dh) has been studied. We characterized the phenotype of Dh, and identified the causative gene of Dh by the methods of positional cloning. The Dh mouse is characterized by tibial hemimelia, oligo or polydactyly of the hindlimbs, asplenia, and urogenital abnormality including imperforate vagina and hydropic kidney. To identify the location of the semi-dominant mutation of Dh, positional cloning method was used. From the linkage analysis, we found that the Dh gene is positioned between D1Mit310 and D1Mit389. Using the conservation of gene order between human and mouse, the 10 candidate genes of Dh were extracted from human genome map. The RT-PCR analysis of candidate genes shows that the mRNA level of one candidate gene is changed. Sequence analysis was performed in the causative gene and its regulatory region from mutant and normal mice and the mutation was identified.

**Genome-wide association study of human narcolepsy; the first and second screening.** *M. Kawashima<sup>1</sup>, T. Ikuta<sup>2</sup>, G. Tamiya<sup>2</sup>, H. Hohjoh<sup>1</sup>, T. Juji<sup>3</sup>, Y. Honda<sup>4</sup>, H. Inoko<sup>2</sup>, K. Tokunaga<sup>1</sup>.* 1) Department of Human Genetics, Graduate School of medicine, University of Tokyo, Tokyo, Japan; 2) Department of Molecular Life Science, Tokai University school of medicine, Kanagawa, Japan; 3) Japanese Red Cross Central Blood Center, Tokyo, Japan; 4) Sleep Disorder Clinic of Seiwa Hospital, Tokyo, Japan.

Narcolepsy is considered to be a sleep disorder involving multiple genetic and environmental factors. A genetic factor strongly associated with the disorder has been found in the human leukocyte antigen (HLA) region: HLA-DRB1\*1501 - DQB1\*0602 haplotype confers strong predisposition to narcolepsy. We performed the genome-wide association study using about 25,000 of microsatellite markers as a tool for identifying disease susceptibility regions. The subjects investigated in this study were all Japanese living in the Tokyo area. One hundred and five narcoleptic patients were pooled for the 1st set and the other 110 samples were pooled for the 2nd set. Similarly, 210 unrelated healthy individuals as controls were pooled for the 1st set and the other 210 samples were pooled for the 2nd set. We used the 1st set pooled DNAs for the primary screening of microsatellite markers and the 2nd set for the second screening to avoid false positive associations. Allele frequencies were estimated from heights of the peaks detected by an automated sequencer (ABI 3700) with GeneScan software. Then, we performed Fishers exact test using 2 x 2 or 2 x m (m; allele number) tables. After the analysis, five markers showed the significant differences in both screenings (p<0.001). These markers again showed strong associations with the disorder in the following analysis using all individual samples. Three candidate regions are now subjected to SNP analysis to further narrow down the susceptibility regions of narcolepsy.

**A genome wide scan in a family with Meckel syndrome suggesting a putative novel locus for MKS, MKS4.** *M.M. Kyttala<sup>1,2</sup>, R. Salonen<sup>3</sup>, R. Karikoski<sup>4</sup>, T. Varilo<sup>1</sup>, M. Kestila<sup>1</sup>, L. Peltonen<sup>1,2</sup>.* 1) Molecular medicine, National Public Health Institute, Helsinki, Finland; 2) Department of Human Genetics, University of California Los Angeles, USA; 3) Prenatal Genetics, Department of Obstetrics and Gynecology, Helsinki University Hospital; 4) Department of Paediatric Pathology, Helsinki University Hospital, Finland.

Meckel Syndrome (MKS) is a lethal autosomal recessive malformation syndrome. In the most typical cases occipital encephalocele with microcephaly, polycystic kidneys, fibrotic changes of liver and postaxial polydactyly are seen. MKS affects 1:9000 births in the Finnish population and was mapped in a Finnish study sample to chr17q23 named as MKS1 (OMIM 249000). 70% of the known Finnish MKS families have been linked to this chromosomal region and most patients share the same haplotype suggesting a common founder mutation in this isolated population. Two other genetic loci for MKS have been identified on chr11q13, MKS2 (OMIM 603194) in Northern African and Middle Eastern populations and on 8q24, MKS3 (OMIM 607361) in consanguineous families from Pakistan and Northern India. We report here a Finnish family with two affected with MKS and two healthy children. Both affected children fulfil the strict diagnosis criteria for MKS and the diagnosis was also confirmed by histological examination of liver. A genealogical study of the family showed evidence of the mothers mothers of both parents originating from the same rare family name on 18th century, and are most likely remote relatives. A linkage analysis excluded the three known MKS loci in the family and therefore we performed a genome wide scan. In two point analysis we obtained the maximum lod scores with supporting p-values ( $p < 0.02$ ) on five chromosomal locations, other than the already known MKS loci. At the moment we are fine mapping and analysing these putative novel genetic loci to confirm the linkage to the genuine MKS4 locus found in this family. This study may provide essential new information about the genetics of MKS and it may also facilitate the identification the disease genes on the other MKS loci, which still remain unidentified.

**Hydrolethalus Syndrome is Caused by a Missense Mutation in a Novel Gene Coding For a Cytoplasmic Protein.**

*L. Lanyi*<sup>1</sup>, *J. Vesa*<sup>1</sup>, *S. Finnila*<sup>1</sup>, *I. Visapaa*<sup>1,2</sup>, *K. Hovanes*<sup>1</sup>, *J. Lee*<sup>1</sup>, *R. Salonen*<sup>3</sup>, *L. Peltonen*<sup>1,2</sup>. 1) Dept Human Genetics, David Geffen School of Medicine at Univ California, Los Angeles, 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 an autosomal recessive lethal malformation syndrome characterized by developmental defects in multiple fetal tissues. The disease is enriched in the Finnish population with an incidence of at least 1:20,000. We earlier mapped and restricted the region to a critical 1cM interval on 11q23-25 using linkage disequilibrium (LD) and shared ancestral haplotype analysis. Here we have utilized LD and haplotype analysis of single nucleotide polymorphism (SNP) markers to further restrict the locus to 470 kb between the gene PREP2 and marker D11S975. A disease associated nucleotide change was identified in one of the predicted transcripts (Celera:hCG1644899, NCBI:FLJ32915) referred to as the HLS gene. This A to G transition in this novel gene (FLJ32915: nucleotide1168) results in an Asp211Gly change in the predicted 299 amino acid polypeptide with no known functional domains. The carrier frequency of the mutation was 2.5% in Eastern Finland, 1.1% in Western Finland, and no carriers were found in the mixed European control panel. The HLS gene shows alternative splicing and is ubiquitously expressed. Wild- type (WT) polypeptides expressed from the cDNA in vitro resulted in diffuse staining of cytoplasm whereas immunostaining of the polypeptides synthesized from mutated cDNA revealed distinct nuclear vesicles. This would imply defective cellular targeting of mutant protein also in vivo.

Program Nr: 1855 from 2003 ASHG Annual Meeting

**Association between schizophrenia and the syntaxin 1a gene.** *A.H.C. Wong<sup>1,3,4</sup>, J. Trakalo<sup>4</sup>, O. Likhodi<sup>4</sup>, M. Yusuf<sup>4</sup>, A. Macedo<sup>5</sup>, M.-H. Azevedo<sup>5</sup>, T. Klemper<sup>3</sup>, M.T. Pato<sup>6</sup>, W.G. Honer<sup>7</sup>, C.N. Pato<sup>6</sup>, H.H.M. Van Tol<sup>1,2,3,4</sup>, J.L. Kennedy<sup>1,3,4</sup>.* 1) Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada; 2) Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada; 3) Institute of Medical Science, University of Toronto, Toronto, Ontario, Canada; 4) Centre for Addiction and Mental Health, Toronto, Ontario, Canada; 5) Department of Psychiatry, University of Coimbra, Portugal; 6) State University of New York, Syracuse, NY; 7) Centre for Complex Disorders, University of British Columbia, Vancouver, BC, Canada.

**Background:** Both microarray and candidate molecule studies have demonstrated that protein and mRNA expression of syntaxin and other genes involved in synaptic function are altered in the cerebral cortex of patients with schizophrenia. **Methods:** DNA from 186 nuclear families with a schizophrenia proband was genotyped for the syntaxin 1a gene at two single-nucleotide polymorphisms, one located in exon 3 and the other in intron 7. Genetic association was analyzed using the transmission disequilibrium test (TDT). **Results:** A significant association was found between schizophrenia and the intron 7 polymorphism ( $p=0.015$ ) and with a haplotype consisting of the two polymorphisms ( $p=0.0069$ ). **Conclusions:** This is the first report of a genetic association between schizophrenia and the STX1a gene in a family-based sample. Combined with gene expression data, this genetic finding strengthens the hypothesis that STX1a is involved in schizophrenia.

**Haplotype tag SNPs in exclusion of the candidate gene, *CBLB*, as a common factor in susceptibility to the complex autoimmune disease, type 1 diabetes.** *F. Payne, H. Rance, B. Barratt, J.A. Todd.* JDRF/WT, DIL, CIMR, Univ of Cambridge, WT/MRC Building, Addenbrooke's Hospital, Cambridge. CB2 2XY.

The Komeda diabetes prone (KDP) rat is a spontaneous animal model of human autoimmune, T-lymphocyte-associated type 1 diabetes (T1D), in which the majority of the disease susceptibility can be attributed to two unlinked loci, the *Iddm/kdp1* locus on rat chromosome 11 and the Major Histocompatibility Complex. Previously, *kdp1* was identified as a nonsense mutation in the T lymphocyte regulatory gene, *Cblb*, which had been identified as an essential negative regulator of T lymphocyte activation. Following the strategy for examining the human orthologues of susceptibility genes identified in animal models for association with T1D, we harvested 35 polymorphisms (30 single nucleotide polymorphisms (SNPs) and five indels) from 11.8 kb of *CBLB* covering exons and 2.5 kb of 5 and 3 sequence, by re-sequencing of PCR products from 96 type 1 diabetic subjects. No non-synonymous variants were observed, nor any obvious candidate for a variant that could change *CBLB* function or expression. From the 21 polymorphisms with allele frequencies 3 %, we selected nine haplotype tag SNPs (htSNPs) that captured the allelic variation of the untyped SNPs with a minimum  $r^2$  of 0.8. These htSNPs were then genotyped in 753 T1D affected sib-pair families from the UK and USA, and the results used to calculate a global multi-locus transmission/disequilibrium test (TDT) that jointly tests the association of all of the variants in the region simultaneously. From the htSNP genotypes and patterns of linkage disequilibrium, genotype data were also imputed for each of the untyped markers and single locus TDT performed. The global multi-locus TDT test gave a *P*-value of 0.69, whereas the smallest single locus TDT *P*-value was 0.06. Although these data suggest that *CBLB* is not a major susceptibility locus in human T1D, the application of htSNPs has enabled the rapid and efficient evaluation of this gene with a 70 % saving in genotyping costs.

Program Nr: 1857 from 2003 ASHG Annual Meeting

**Ankyrin and SOCS box containing gene 2 (ASB2) in a linked region on 14q32 is associated with asthma and bronchial hyperresponsiveness (BHR) in the Hutterites.** *Z. Tan, D. Schneider, D. Newman, R. Stanaker, C. Ober.* Department of Human Genetics, The University of Chicago, Chicago, IL.

A broad region on chromosome 14q24-q32 was linked to asthma in at least 4 different populations, including the Hutterites (Ober et al., 2000; *Am J Hum Genet* 67:1154) and families in the Collaborative Study on the Genetics of Asthma (CSGA) (Xu et al., 2001; *Am J Hum Genet* 68:1437). In these two populations, there was evidence for linkage at D14S749 at 97.7 cM from p-ter (Marshfield Map). We searched the public databases for gene(s) near D14s749 and identified ASB2, which has a Suppressor of Cytokine Signaling (SOCS) box and is involved in cell growth and differentiation, as an excellent candidate. We genotyped 693 members of a large Hutterite pedigree for 3 SNPs located approximately 7 kb apart, in intron 4, intron 5, and the 3'UTR (minor allele frequency 0.33, 0.32, 0.32, respectively). We tested each SNP and pairwise combinations of the SNPs for association by the TDT. Significant overtransmission at each of the three loci ( $P=0.005-0.011$ ) was observed for individuals with asthma (defined by the presence of BHR and symptoms). Pairwise combinations of SNPs spanning the gene were also significantly over- and undertransmitted to individuals with BHR ( $P<0.01$ ), suggesting that an ASB2 haplotype or multiple SNPs within the gene contribute to susceptibility. Additional genotyping of variation in ASB2 as well as in neighboring genes is underway. Nonetheless, these data suggest that variation in ASB2, or variation in linkage disequilibrium with an ASB2 haplotype, is associated with asthma and may account for some of the linkage to 14q32 that was observed in the Hutterites. Supported by HL56399.

Program Nr: 1858 from 2003 ASHG Annual Meeting

**A SNP map of human chromosome 20q12-13.1.** *J.L. Bento, J.C. Mychaleckyj, B. Roh, P. Hicks, B.I. Freedman, D.W. Bowden.* The Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC.

Human chromosome 20q12-13.1 is linked to type 2 diabetes mellitus (T2DM) in multiple studies. To identify T2DM susceptibility gene(s) in this region, a SNP fine map has been constructed across a 6 Mb region (41.6-47.5 Mb), containing the candidate diabetogenic genes, adenosine deaminase (ADA), hepatocyte nuclear factor 4 alpha (HNF-4) and glucose transporter 10 (GLUT-10). Verified SNPs from the NCBI dbSNP database, with minor allele frequency greater than 0.20, were placed on the map at an average density of 1 SNP per 25 kb. A total of 116 SNPs were genotyped on 310 Caucasian controls and 300 Caucasian cases with T2DM and end stage renal disease, using the Sequenom MassArray genotyping system. SnpAnalyzer, a web-based, enhanced version of the SnpAnalysis package was used to test each SNP for Hardy-Weinberg equilibrium, and to calculate inter-SNP linkage disequilibrium. Within regions of high LD ( $D > 0.70$ ), haplotypes were reconstructed using PHASE and parametric and non-parametric tests for association with T2DM performed using Excel and Clump.

Currently, 1.142 Mb of the region falls into 9 haplotype blocks of high inter-SNP LD (range=64-223 kb, mean=127 kb) but there are also extensive regions that lie outside the blocks, the largest being 259 kb. An additional set of SNPs is being genotyped in regions of low SNP density. Seven SNPs show evidence of allelic association with T2DM ( $p < 0.05$ ), with the strongest association centered on an intronic SNP in the gene cadherin-like 22 (CDH22,  $p = 0.00146$ ). Each SNP was also genotyped on 40 CEPH trios, and showed similar LD results and haplotype structures to the Caucasian population of unrelateds. These results indicate that the haplotype structure of the CEPH individuals is comparable to our Caucasian population, and therefore the CEPH trios offer a powerful independent method to confirm or predict block structure, with the benefit of inheritance-based ambiguous phase resolution. In conclusion, this SNP map will provide a framework for our on-going T2DM susceptibility gene association study, and for other diseases that map to this region.

Program Nr: 1859 from 2003 ASHG Annual Meeting

**Linkage Disequilibrium Surrounding the *CDKN1A* Gene.** *L. Geller, G.P. Larson, C. Ouyang, T.G. Krontiris.*  
Division of Molecular Medicine, Beckman Research Institute of the City of Hope, Duarte, CA, 91010.

We analyzed genetic variation in a 28-kb region surrounding the *CDKN1A* gene, located at 6p21. *CDKN1A* encodes a 21-kd cyclin dependent kinase inhibitor p21<sup>*Waf1/Cip1*</sup>, which is believed to be a major downstream effector of p53 in cell cycle regulation. Although polymorphisms within the coding region are rare (we have found only the Codon-31<sub>SerArg</sub> variant), we identified 87 SNPs, insertions, and deletions upstream of the gene and within the promoter, introns, and 3' UTR. These polymorphisms are distributed over at least 20 haplotypes apparently resulting from crossovers and gene conversions. Some of these SNPs and haplotypes maybe associated with cancer risk. This region is characterized by high levels of extended, overlapping linkage disequilibrium (LD) that is distributed over several haplotypic backgrounds and includes two complex microsatellite markers downstream. Meiotic recombination at potential recombination hotspots near *CDKN1A* is currently under investigation.

**Linkage disequilibrium and haplotype mapping of the human cytoplasmic dynein heavy chain 1 gene on chromosome 14q32.** A. Ahmad-Annuar<sup>1</sup>, P. Shah<sup>1</sup>, M. Hafezparast<sup>1</sup>, D.B. Goldstein<sup>2</sup>, E.M.C. Fisher<sup>1</sup>. 1) Department of Neurodegenerative Disease, Institute of Neurology, London, United Kingdom; 2) Department of Biology, University College London, United Kingdom.

Cytoplasmic dynein heavy chain 1 (*Dnchc1*) has recently been shown to be the causal gene in a mouse model of motor neuron degeneration, Legs at Odd Angles (*Loa*). The *Loa* mouse displays progressive locomotor deficits and pathological features similar to aspects of human motor neuron disease, including loss of anterior horn cells and presence of inclusion bodies positive for SOD1, ubiquitin, CDK5 and neurofilaments. *Dnchc1* is part of the dynein-dynactin pathway which is integral in maintaining retrograde transport in neurons and microtubule-associated activities, and these functions have been shown to be perturbed in the *Loa* mice. The human homologue for the mouse *Dnchc1* lies on chromosome 14q32. We have defined the structure of the human *DNCHC1* and found it to be a large gene with 78 exons spanning 86kb. Mutational analysis of *DNCHC1* in patients with motor neuron disease, of the exon known to be mutated in *Loa* mouse, did not reveal any changes associated with the disease. Due to the size of *DNCHC1*, we have undertaken the haplotype mapping approach to facilitate the study of the gene. Statistical haplotyping of 16 single nucleotide polymorphisms (SNPs) within the human *DNCHC1* locus in 32 CEPH trios was performed to elucidate the haplotype block structure across this region. Linkage disequilibrium was estimated using  $D'$  values and revealed a pattern of two distinct haplotype blocks across *DNCHC1*. Haplotype diversity was found to be distinguishable by three tagging SNPs, however *DNCHC1* demonstrated lower haplotype diversity in the CEPH population than expected. Future work involves analysing the *DNCHC1* locus in other populations for comparison against the CEPH haplotypes as well as screening a large collection of patients with motor neuron disease to find an association between haplotype and phenotype.

**No support for association of the DTNBP1 (dysbindin) gene with schizophrenia in large samples from Germany, Poland, and Sweden.** *S. Cichon*<sup>1</sup>, *A. Van Den Bogaert*<sup>1</sup>, *A.C.J. Otte*<sup>2</sup>, *T.G. Schulze*<sup>3</sup>, *J. Schumacher*<sup>2</sup>, *S. Ohlraun*<sup>3</sup>, *P. Czerski*<sup>4</sup>, *J. Hauser*<sup>4</sup>, *E. Jönsson*<sup>5</sup>, *W. Maier*<sup>6</sup>, *P. Propping*<sup>2</sup>, *M. Rietschel*<sup>3</sup>, *M.M. Nöthen*<sup>1</sup>. 1) Dept Medical Genetics, Univ Antwerp, Antwerp, Belgium; 2) Inst Human Genetics, Univ Bonn, Bonn, Germany; 3) Central Inst Mental Health, Mannheim, Germany; 4) Dept Psychiatry, Univ Poznan, Poznan, Poland; 5) Dept Clinical Neuroscience, Psychiatry Section, Karolinska Inst, Stockholm, Sweden; 6) Dept Psychiatry, Univ Bonn, Bonn, Germany.

Recently, Straub et al. (2002) identified significant associations between single nucleotide polymorphisms (SNPs) within the positional candidate gene DTNBP1 (dystrobrevin-binding protein 1, or dysbindin) on 6p22.3 and schizophrenia in the Irish population. In a subsequent replication study, Schwab et al. (2003) tested six SNPs in two independent parent-offspring trio samples from Germany and obtained confirmatory evidence for association. A second replication study by Morris et al. (2003) investigated a case-control sample of schizophrenia from the Irish population. No evidence was found to suggest an association between the dysbindin gene and schizophrenia in their sample. The major difference between their study and the reports by Straub et al. (2002) and Schwab et al. (2003) was that the case-control sample used by Morris et al. (2003) was not selected for familiarity. We have performed an independent replication study in a large sample of 418 schizophrenic cases from Germany that were not selected for familiarity and 284 controls using SNPs in the dysbindin gene. Two other, smaller case-control samples were tested, one from Sweden (158 cases/292 controls) and one from Poland (296/115). No significant p-values were observed in single-marker and haplotype analyses. Likewise, no significant p-values were observed in a separate analysis of the subsamples with a positive family history of schizophrenia and other psychiatric disorders. Our results are in line with the finding by Morris et al. (2003) and might reflect different genetic mechanisms underlying individuals derived from high-density families and cases coming from families less heavily loaded with schizophrenia.

**SNP map and haplotype analysis of the beta-globin gene region in beta<sup>0</sup>-thal/HbE patients.** *K.J. Abel<sup>1</sup>, J. Whitacre<sup>1</sup>, O. Sripichai<sup>2</sup>, P. Erlich<sup>3</sup>, L. Farrer<sup>3</sup>, A. Braun<sup>1</sup>, S. Fucharoen<sup>2</sup>.* 1) SEQUENOM, Inc., San Diego, CA; 2) TRC, Mahidol University, Nakornpathom, Thailand; 3) Boston University, Boston, MA.

Beta-thalassemias are extremely common in SouthEast Asia, and due to recent worldwide migrations these abnormal alleles and associated disorders are becoming more common in other regions. Many genetic defects responsible for beta thalassemias have been described. The mutant HbE allele encodes a Glu to Lys amino acid substitution at codon 26, plus the potential for alternative mRNA splicing leading to a frame shift mutation. A puzzling feature of patients compound heterozygous for beta<sup>0</sup>-thalassemia/HbE is the variable disease presentation, ranging from nearly asymptomatic to severe, transfusion-dependent disease. The need exists to identify the modifying genetic factors, representing either novel therapeutic targets or the focus of new diagnostics to identify at-risk individuals for early intervention.

Using an automated, chip-based platform for genetic analysis based on mass spectrometry (MassARRAY), we are examining the extent to which genetic variation within the beta globin gene complex may contribute to thalassemia disease severity in Thai/Chinese patients with beta<sup>0</sup>-thalassemia/ HbE disease. Toward this goal a map of single nucleotide polymorphisms (SNPs) has been constructed spanning more than 80 Kb, including all beta-like genes and extending into the locus control region (LCR). Assays were designed for >130 putative SNPs obtained from targeted resequencing efforts and from the public domain, and are being tested in individuals from different ethnic groups. Also included are assays for polymorphic sites reported to influence globin gene expression (XmnI<sup>+/-</sup> and BP1-binding sites upstream of  $\alpha$ - and  $\beta$ -globin, respectively). SNPs having minor allele frequencies >0.15 are being selected at spacings of roughly 2-3 Kb. Multiplexed assays are being used in haplotype analyses for association with disease severity. These efforts will be complemented by a genome scan for other modifier alleles, comparing up to 100,000 gene-based SNP frequencies in pooled DNAs from mild and severe disease patients.

**Haplotype structure of *ESRI* and association studies with complex traits.** *J. Butler*<sup>1</sup>, *P. Bretsky*<sup>2</sup>, *C. Haiman*<sup>2</sup>, *D.O. Stram*<sup>2</sup>, *M.C. Pike*<sup>2</sup>, *K.L. Lunetta*<sup>3</sup>, *K.G. Ardlie*<sup>3</sup>, *B.E. Henderson*<sup>2</sup>, *M.J. Daly*<sup>4</sup>, *D. Altshuler*<sup>4,5</sup>, *J.N. Hirschhorn*<sup>1,4</sup>. 1) Genetics, Children's Hospital/Harvard Med School, Boston, MA; 2) Norris Comprehensive Cancer Center/Keck School of Med, USC, Los Angeles, CA; 3) Genomics Collaborative, Cambridge, MA; 4) Whitehead/MIT Center for Genome Research, Cambridge, MA; 5) Genetics/Medicine, Mass General Hospital/Harvard Med School, Boston, MA.

In haplotype-based association studies, single nucleotide polymorphisms (SNPs) are used to determine the particular patterns of alleles (haplotypes) that are common in the population. A set of haplotype-tagging SNPs (htSNPs) is chosen to assay the common haplotypes and the variants carried on these haplotypes, allowing more efficient and comprehensive studies. We are applying this approach to studies of *ESRI*, encoding estrogen receptor. We chose to study *ESRI* because it is an excellent candidate for two traits of interest to us, breast cancer and stature, and is in a region that we and others have linked to stature (6q24-25).

To determine common haplotypes (>5% frequency) within blocks in *ESRI*, we genotyped SNPs in 349 unrelated individuals from 5 self-described ethnic groups: African-American, White, Hawaiian, Japanese, and Latino. Within blocks of strong linkage disequilibrium (LD), custom software packages were used to reconstruct haplotypes and select htSNPs.

We successfully genotyped 140 SNPs in 480 kb encompassing *ESRI* and its known promoters. Consistent with previous studies, we observed blocks of strong LD with generally low haplotype diversity (3-6 common haplotypes/block). We identified a set of approximately 60 htSNPs for the non-African-derived populations. At least 20 additional htSNPs were required to capture the common haplotypes in African-Americans. By genotyping the htSNPs in 1500 people from each end of the stature distribution, and in a large (n = 3936) case-control study for breast cancer, we are testing *ESRI* for a role in these complex traits. The htSNPs we identified should also be useful in studies of *ESRI* with other complex traits and in a wide variety of ethnic groups.

**Identification of framework haplotypes defining blocks of linkage disequilibrium conserved across ethnic populations.** *C. Ouyang, T.G. Krontiris.* Division of Molecular Medicine, Beckman Research Institute of the City of Hope, Duarte, CA.

The recent discovery of a block-like haplotype structure of linkage disequilibrium (LD) throughout the human genome has sparked hope for the future success of genome-wide disease mapping studies. In a study designed to determine the required single nucleotide polymorphism (SNP) density to uncover major underlying haplotypes within LD blocks, we analyzed the haplotype diversity of 13 genes distributed across the length of the long arm of chromosome 5 and spanning 247 kb at single-base resolution. We found that haplotype diversity could generally be simplified into one to three frameworks by using highly structured SNPs conserved across different human populations. These framework SNPs could generally be easily identified as having allele frequencies greater than 15%; and pairwise  $r$  near 1. Our analyses suggested that such framework haplotypes were likely to be the common ancestral backgrounds upon which more recent mutations were superimposed. Nucleotide diversity across a given region was directly correlated with the number of framework haplotypes. At some loci, we observed a reduction of frameworks in individuals of European descent when compared to individuals of African descent, supporting the out-of-Africa scenario. Preliminary studies at 6 other loci not on chromosome 5 revealed the same patterns. We discuss the advantages using our framework haplotype analysis to define the boundaries of LD blocks and to simplify haplotype diversity for genome-wide association studies.

**SNP frequency, haplotype structure and linkage disequilibrium in human FBN1-gene.** *C. Kraus, M. Krumbiegel, A. Reis.* Institute of Human Genetics, University of Erlangen, Germany.

The fibrillin gene (FBN1) is a relatively large (approximately 100kb) and highly fragmented (65 exons) gene. Mutations in the FBN1 cause Marfan syndrome and autosomal dominant Weill-Marchesani syndrome. Moreover, it is a candidate for association studies in complex disease. To determine the SNP frequency and haplotypes structure of the human FBN1 gene we analyzed a total of 28 single-nucleotide polymorphisms (SNPs) spanning the entire 110kb locus. 17 of these 28 SNPs were initially identified as sequence variants in Marfan patients, of which 8 were already published in the database. Additionally, 11 SNPs were selected from the genome SNP database (dbSNP), whereas heterozygosity of 0.3 was the major criteria. SNPs were genotyped on 96 unrelated individuals from Caucasian origin by sequencing the corresponding fragments. For the analysis for the linkage disequilibrium (LD) and haplotypes structure of the region we chose those SNPs, which showed a minor allele frequency of at least 9% in our control group. Only 18 of the 28 SNPs analyzed fulfilled this criteria. Of the published 11 SNPs with a given heterozygosity of 0.3, 3 were not detected in our control samples and two were found only twice. LD was calculated with *ldmax* using an expectation-maximization algorithm and visualized with *GOLD*. SNPs in a region of 110kb covering the entire FBN1 locus are in a significant pair wise LD. Haplotypes and their frequencies were estimated using the *PHASE* program. Within this region 5 common haplotypes were observed accounting for 91% of chromosomes. The most frequent one was found in 64% of the control samples. All but two of the rare haplotypes can be reconstructed from the 3 most common by changing only one site. To review the quantity of chromosomes which are necessary for definition of LD and haplotypes structure of the FBN1 locus, calculation were repeated with 48 control persons. Repeated calculation with only 48 control persons demonstrated the same LD and haplotype structure.

**Linkage disequilibrium analysis of single nucleotide polymorphisms of the glutamate receptor 6 gene and schizophrenia.** *M. Martinez*<sup>1</sup>, *A.R. Sanders*<sup>2</sup>, *L. Martinolich*<sup>2</sup>, *E.B. Carpenter*<sup>2</sup>, *J. Duan*<sup>2</sup>, *B.J. Mowry*<sup>3</sup>, *D.F. Levinson*<sup>4</sup>, *R.R. Crowe*<sup>5</sup>, *J.M. Silverman*<sup>6</sup>, *P.V. Gejman*<sup>2</sup>. 1) INSERM EMI 00-06, Evry, France; 2) Schizophrenia Genetics Research Program Univ of Chicago, Chicago, IL; 3) Center for Schizophrenia Research, Univ of Queensland, Brisbane, Australia; 4) Dept of Psychiatry, Univ of Pennsylvania School of Medicine, PA; 5) Dept of Psychiatry, Univ of Iowa, Iowa City, IA; 6) Dept of Psychiatry, Mount Sinai School of Med, New York, NY.

The chromosome 6q13-q26 region has been implicated as harboring a schizophrenia susceptibility gene (SCZD5) in multiple independent family collections. Interestingly, the 6q21 region has also been described as a candidate region for autism. A positive association has recently been reported with the glutamate receptor 6 (GRIK2) gene and this syndrome. Glutamate is the principal excitatory neurotransmitter in the brain and is directly involved in cognitive functions such as memory and learning. We report here on a linkage disequilibrium analysis of 13 GRIK2-SNPs in a sample of 146 schizophrenia pedigrees (>750 subjects with DNA). Using family-based tests in the FBAT program, each SNP was tested for association with SZ individually and by multilocus analysis. No significant association was found. The most significant results were observed with G-14810e2A and G-2710e14T (p=0.20). Altogether, our results do not indicate that GRIK2 gene is a strong candidate gene for SZ.

**Simulating a Coalescent Process with Disease Ascertainment Bias.** *Y. Wang, B. Rannala.* Medical Genetics, University of Alberta, Edmonton, Alberta, Canada.

There is currently much interest in the possibility of using population linkage disequilibrium (LD) to identify and localize disease-associated mutations on chromosomes. One approach to evaluating the potential power and feasibility of these LD mapping methods is to simulate population samples of chromosomes carrying linked genetic markers to examine the expected extent of LD. To do so, new simulation methods are needed that take account of genetic and population demographic processes as well as the sample ascertainment bias present in case-control studies of genetic diseases. A new method is presented for use in simulating samples of disease and normal chromosomes bearing multiple linked genetic markers under a neutral model of mutation, genetic drift, and recombination. The basic strategy is to simulate the sample path (over time) of the population frequency of a disease allele, using a diffusion approximation, and then to simulate the coalescent of a sample of chromosomes conditional on the sample path of the allele frequency. The method is implemented in a computer program. We evaluated the simulation method by comparing the results with a real data set used to positionally clone the diastrophic dysplasia (DTD) gene. The pattern of LD is similar between the DTD data and the simulated data. We also applied the simulation program to study the general effects of disease mutation age (frequency), marker allele polymorphism cutoff level, and sample size, on the pairwise LD. It is shown that the levels of LD between markers and a disease mutation is lower for older, or more prevalent, disease mutations. The marker polymorphism level also has an important effect on LD. The results suggest that selecting marker loci with higher polymorphism levels may increase the power of LD mapping. This effect decreases with an increasing the map distance of the marker from the disease mutation. The level of LD may be biased upward with small sample sizes. If more chromosomes are sampled (more than 100), the average LD changes little.

**Investigation of recombination hotspots and blocks of linkage disequilibrium within the human *PGMI* gene: An allelic association and mapping approach.** *N.A Rana*<sup>1, 2</sup>, *N.D. Ebenezer*<sup>1</sup>, *A.R. Webster*<sup>1</sup>, *D.B. Whitehouse*<sup>2</sup>, *S. Povey*<sup>2</sup>, *A.J. Hardcastle*<sup>1</sup>. 1) Inst.of Ophthalmology, UCL, UK; 2) The Galton Laboratory, UCL, UK.

The distribution of linkage disequilibrium (LD) in the human genome has important consequences for the design of experiments that infer susceptibility genes for complex disease using association studies. Recent studies have shown a non-random distribution of human meiotic recombination associated with intervening tracts of LD. This phenomenon can be better understood by the continual dissection and fine structure analysis of several genomic regions. Here, we report clustered hotspot activity with intervening blocks of LD within the human *PGMI* gene (1p31) using data derived from comparative mapping, meiotic and population studies. Earlier work has suggested a high recombination rate in two regions within the *PGMI* gene; Site A (exons 4 to 8) and Site B (exons 1A to 4). Site A has been previously studied, Site B is the subject of this current study. CRIMAP was used to construct a genetic map of the region using locally typed intragenic *PGMI* markers and previously typed CEPH markers. Integration of this map with physical data revealed the *PGMI* recombination rate to be 50 times higher than the genomic average. Specific recombination events in CEPH families and large multi-generation families were sought and further refined. The recombination rate across *PGMI* was estimated at 37.8 cM/Mb showing an excess of male recombinants (12.6 cM/Mb female, 63.1 cM/Mb male,  $p=0.10$  two-tailed). Sequencing of 8 individuals across 6 Kb of targeted regions in Site B identified 18 informative SNPs. Individuals from three distinct populations, Caucasian ( $n=264$ ), Chinese ( $n=222$ ) and Vietnamese ( $n=187$ ) were genotyped, and haplotypes determined using EH, Idmax and Arlequin. Allelic association and haplotype analysis in these samples revealed variable recombination rates in the region, demonstrating the presence of, (i) at least two hotspots and, (ii) two haplotype blocks. The spatial arrangement of haplotype blocks was identical in all three populations. Fine mapping of recombination events placed these at the regions of LD collapse. Sequence motifs associated with recombination will also be presented.

**Marker-dependent background disequilibrium on human chromosome 11p.** *L. Rodríguez<sup>1</sup>, J. Hermida<sup>1</sup>, E. Sande<sup>1</sup>, S. Rodríguez<sup>1</sup>, C. Nuñez<sup>1</sup>, C. Louro<sup>1</sup>, C. Martín<sup>2</sup>, C. Zapata<sup>1</sup>.* 1) Departamento de Genética, Universidad de Santiago, 15782 Santiago de Compostela, Spain; 2) Departamento de Dermatología y Otorrinolaringología, Universidad de Santiago, 15782 Santiago de Compostela, Spain.

Information on the amount and distribution of gametic disequilibrium across human genome (background disequilibrium) is relevant to successful localization of disease genes by disequilibrium. Background disequilibrium over large anonymous regions of human chromosomes has been evaluated using genetic markers with very different evolutionary dynamics, such as microsatellites and SNPs. It follows that quantification of the amount of background disequilibrium can be seriously confounded by the occurrence of marker-dependent disequilibrium. Here, we report a study aimed at assessing the influence of different genetic markers on the quantification on background disequilibrium across an extensive anonymous region of human genome. Specifically, disequilibria between microsatellites and SNPs distributed along the human chromosome 11p were compared, from two independent large samples of the Galician population (northwest Spain). Our observations show that the amount of disequilibria is similar for these two genetic markers but their patterns of distribution along 11p differ markedly. Notably, disequilibria between microsatellites extend over wider regions than disequilibria between SNPs. In addition, a significant negative correlation was detected between the strength of disequilibrium between SNPs and recombination frequency. In contrast, the strength of disequilibrium between microsatellite pairs does not appear to be correlated with recombination frequency. Our findings suggest that the occurrence of marker-dependent background disequilibrium is a relevant factor for designing and interpreting experiments of localization of disease genes by disequilibrium mapping.

**Characterization and comparison of multiple pair wise linkage disequilibrium indices and definitions of haplotype blocks using simulated SNPs data.** *A. Takahashi<sup>1</sup>, R. Yamada<sup>2</sup>, T. Tsunoda<sup>1</sup>*. 1) Laboratory for Medical Informatics, SNP Research Center, The Institute of Physical and Chemical Research(RIKEN); 2) Laboratory for Rheumatic Diseases, SNP Research Center, The Institute of Physical and Chemical Research(RIKEN).

It is well recognized that linkage disequilibrium (LD) mapping and haplotype block analysis using SNPs are promising. There are, however, multiple indices for quantification of pair wise LD and definitions of haplotype blocks are also multiple. These indices of LD and definitions of haplotype blocks have been used in various studies without good characterization of them from the standpoint of genome-wide evaluation with SNPs. It seems necessary that relationship between different LD indices and haplotype blocks by different methods should be investigated to offer basic data for various genetic studies. For this purpose, we created simulation data of haplotypes consisted of SNPs by considering recombinations, genetic drifts and mutations and characterized LD indices and haplotype blocks as well as relationship between LD indices and haplotype blocks.

**Evaluation of different haplotype block definition methods and characterisation of the genomic architecture of human chromosome 17q.** *E. Zeggini*<sup>1</sup>, *A. Barton*<sup>2</sup>, *S. Eyre*<sup>2</sup>, *D. Ward*<sup>2</sup>, *J. Worthington*<sup>1,2</sup>, *S. John*<sup>1</sup>. 1) CIGMR, University of Manchester, Manchester, UK; 2) arc Epidemiology Unit, University of Manchester, Manchester, UK.

The finding of mosaic patterns of linkage disequilibrium (LD) in the human genome have instigated attempts to characterise the distribution of haplotype blocks as a useful tool in association studies. Several recent reports have focused on determining the genomic architecture of different chromosomal segments, each by applying distinct haplotype block definitions based on LD measures or haplotype diversity. The effect that different haplotype block definition methods have on association studies, however, remains unclear. A proposed strategy to utilise blocks in association studies is to initially select haplotype tagging single nucleotide polymorphisms (htSNPs) in the region of interest. We have applied various sets of criteria in characterising blocks in a 6.1Mb region of human chromosome 17q. One hundred eighty nine unrelated healthy individuals were genotyped for 137 SNPs, at a median spacing of 15.5 kb. Haplotype block maps of the region were constructed using diverse methods, including those employed by Gabriel et al., 2002, Daly et al., 2001, Wang et al., 2002, and novel methods we have developed. HtSNPs were identified for each map. Blocks were generally found to be shorter (16464 to 28410 bp) and coverage of the region limited (5% to 12.7%) with definitions based on LD measures, compared to methods based on haplotype diversity (42024 to 130678 bp and 26.2% to 85.8%). Although the distribution of blocks was highly variable, the number of htSNPs was consistent, indicating that, for the marker spacing used in this study, choice of haplotype block definition is not important when used as an initial screen of the region to identify htSNPs. However, methods based on haplotype diversity may have greater utility when used in association studies due to greater coverage of the region, maximising the chances of detecting an association within the boundaries of a block.

**Linkage disequilibrium analysis of G72 in Canadian families with schizophrenia.** *B. Xu*<sup>1</sup>, *E.W.C. Chow*<sup>2</sup>, *A.S. Bassett*<sup>2</sup>, *L.M. Brzustowicz*<sup>1,3</sup>. 1) Dept. of Genetics, Rutgers University, Piscataway, NJ; 2) Dept of Psychiatry, University of Toronto, and Schizophrenia Research Program, Queen Street Division, Centre for Addiction and Mental Health, Toronto, Ontario; 3) Dept of Psychiatry, UMDNJ, Robert Wood Johnson Medical School, Piscataway, NJ.

A number of studies have suggested the existence of a schizophrenia-susceptibility gene on 13q22-34. We have previously described a significant linkage signal on 13q32 in a sample of 22 medium-sized Canadian families selected for study because multiple relatives were clinically diagnosed with schizophrenia or schizoaffective disorder (Brzustowicz et al 2000). Families were analyzed under autosomal dominant and recessive models, with broad and narrow definitions of schizophrenia. All models produced positive LOD scores with markers on 13q. Chumkov et al (2002) have reported linkage disequilibrium between markers in the gene G72, located on chromosome 13q33, and schizophrenia, suggesting this as a candidate gene for schizophrenia susceptibility. In order to investigate if linkage disequilibrium to G72 exists in our sample exhibiting linkage to 13q32, we tested 5 single-nucleotide polymorphism (SNPs) that span the G72 gene, described by Chumkov et al. The original 22 families plus two new families were genotyped by pyrosequencing at these 5 SNPs (rs3916965, rs3916967, rs778293, rs3918342, rs1421292). Evidence for LD was first assessed using the program PSEUDOMARKER which, in contrast to popular TDT-based programs, explicitly separates the evidence for linkage and LD in families with multiple affecteds. Genotypes were analyzed under dominant and recessive pseudomarker models, using both the narrow and broad diagnostic classification. Only one marker, rs3918342, produced evidence for linkage disequilibrium at  $p < 0.05$  ( $p = 0.011$  under broad recessive model), although it is not clear that this result should be considered significant given the multiple markers and models tested. Analysis of 5-marker haplotypes using the program TRANSMIT also failed to identify significant genetic association to schizophrenia. These results suggest that G72 is not the chromosome 13q schizophrenia susceptibility locus acting in these families.

**Large Scale SNP Genotyping at the BCM-HGSC with Molecular Inversion Probe (MIP) Technology.** *F. Yu<sup>1</sup>, G. Scott<sup>1</sup>, X. Lu<sup>1</sup>, D. Nelson<sup>1</sup>, J. Belmont<sup>1</sup>, D. Steffen<sup>1</sup>, S. Leal<sup>1</sup>, P. Hardenbol<sup>2</sup>, M. Thomas<sup>2</sup>, M. Jain<sup>2</sup>, M. Faham<sup>2</sup>, T. Willis<sup>2</sup>, R. Gibbs<sup>1</sup>.* 1) Human Genome Sequencing Center, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX77030; 2) ParAllele Bioscience, 384 Oyster Point Blvd, Suite 8, South San Francisco, California, 94080.

Linkage strategies that have worked well for single-gene Mendelian disorders lack power to map polygenic susceptibility loci. Thus, little is known about the genetic basis of the complex human diseases, such as diabetes, cancer, stroke, Alzheimer's disease, Parkinson's disease, psychiatric disorders, alcoholism, heart disease, deafness, arthritis, and asthma. The goal of this International HapMap Project is to develop a genome-wide haplotype map by identifying the haplotype blocks and the common haplotypes in the human genome, and to define a set of tag SNPs, using the population samples collected from the Yorubas in Nigeria, Japanese, Han Chinese and U. S. residents with ancestry from northern and western Europe. This effort aims at speeding the discovery of genes related to common complex illnesses. In Collaboration with ParAllele, the Human Genome Sequencing Center (HGSC) at BCM in particular is responsible for genotyping the single nucleotide polymorphisms (SNPs) and generating the haplotype map of chromosome 12. We will carry out 60,000 SNP genotyping on 288 individuals in two years. The highly multiplexed genotyping technology, Molecular Inversion Probe (MIP) technology, invented by the scientists at ParAllele is used to perform SNP genotyping. The oligonucleotide probe in this process undergoes a unimolecular rearrangement from a molecule that cannot be amplified into a molecule that can be amplified. HGSC has already generated 1,091,697 genotypes on the 30 CEPH trios.

**Fine Mapping a Gene For Severe Pediatric Gastroesophageal Reflux On Human Chromosome 13q14.** *J. Donfack, F.Z. Hu, A. Amed, R. Dopico, J.C. Post, G.D. Ehrlich, R.A. Preston.* Center for Genomic Sciences, Allegheny General Hospital, Pittsburgh, PA.

A genome-wide screen was used to establish linkage in multiple families between severe pediatric gastroesophageal reflux disease (GERD) and a locus (GERD I) on Chromosome 13q14 (Hu et al., 2000a). Initial refinement of this locus identified a 9 cM interval flanked by the markers CAGR1 (MAB21L1) and D13S263 (Hu et al. 2000b). In a further attempt to reduce the size of the GERD I locus, we have performed linkage disequilibrium (LD) analyses using two sets of DNA specimens. The first study was performed as case/control association study in which we analyzed 73 individual cases and 93 controls, all of white European ancestry. In the second study we performed transmission disequilibrium testing on 22 small families. In both studies all individual were genotyped for forty-one single nucleotide polymorphisms (SNPs) which were selected from among 164 SNPs we had previously identified as part of our ongoing effort to identify the causative mutation for GERD in the five families that were originally used for our linkage study. In the current analyses, we used Chi-Square and the Fischer Exact Tests for the case/control association studies and LD was estimated using the Transmission Disequilibrium Test (TDT) in the family samples. In the family samples TDT analyses revealed preferential transmission of SNP142 ( $3.0 \times 10^{-3}$ ) and the two-marker haplotype SNP160-SNP142 to affected children ( $8.05 \times 10^{-6}$ ). The case/control samples showed significant difference with SNP180 ( $1.0 \times 10^{-3}$ ) and haplotypes SNP32-SNP160 and SNP5-SNP180 ( $4.0 \times 10^{-4}$ ). The combined results from the familial and case/control association tests suggest that the GERD I gene is most likely located between SNP160 and SNP180, a region of about 1.5 Mb.

**Fine linkage mapping and haplotype association localize a susceptibility gene for prostate cancer to a chromosome 3 region bearing the FHIT gene.** *Y. Ding<sup>1</sup>, G.P. Larson<sup>1</sup>, L. Cheng<sup>2</sup>, C. Lundberg<sup>1</sup>, V. Gagalang<sup>1</sup>, G. Rivas<sup>1</sup>, C. Ouyang<sup>1</sup>, L. Geller<sup>1</sup>, T.G. Krontiris<sup>1</sup>, The ECOG E1Y97 Study Group.* 1) Molecular Medicine and; 2) Information Sciences, Beckman Res Inst City of Hope, Duarte, CA.

We performed a candidate gene-based linkage study on 228 affected brother pairs (200 families) to detect susceptibility loci for prostate cancer. Evidence of linkage was initially identified for two candidate genes, CDC25a and FHIT, on chromosome 3. Subsequent fine mapping yielded a maximum LOD score of 3.17 at D3S1234 using LODPAL (S.A.G.E 4.2). For a subset of 38 families (66 pairs), in which three or more affected brothers were reported, the LOD score was elevated to 3.83 at D3S1234. The linkage was also confirmed using MERLIN (Maximum Zmean = 2.94,  $p = 0.002$  at D3S2420). In the subgroup, although maximum linkage peaks produced using S.A.G.E and MERLIN were 10 cM apart, both programs generated maximum sharing of 2 IBD = 0.49 and minimum sharing of 1 IBD = 0.27 at D3S1234, suggestive of a recessive model. We then genotyped 12 SNPs spanning approximately 400 kb surrounding D3S1234 for 162 cases of European descent (one case from each family) and 120 unrelated European-descent controls. We predicted SNP pair-wise haplotypes combining cases and controls using PHASE. We found significant evidence for association between case status and haplotype at SNP pair rs760317/rs722070 (Pearson's  $\chi^2 = 10.76$ ,  $df = 3$ ,  $P = 0.013$ ). The association was particularly strong for a subset of cases (61) that shared 2 alleles IBD with one or more affected brothers. In this subset, haplotype 2/1 was elevated at 56.6% (69/122), compared to 50.3% (163/324) in all cases and 43% (102/240;  $\chi^2 = 9.53$ ,  $df = 3$ ,  $P = 0.023$ ) in controls. Consistent with a recessive inheritance, the frequency of 2/1 homozygotes was 32.8% (20/61) in the 2 IBD subset, compared to 25.7% (26/101) in the remaining cases and only 16.7% (20/120;  $\chi^2 = 6.105$ ,  $df = 1$ ,  $P = 0.014$ ) in controls. Our results strongly suggest a new locus related to prostate cancer risk on chromosome 3p14.2, a region frequently involved in chromosomal instability in many types of cancer. These findings warrant further confirmation through extended linkage and association studies.

**Mapping of an Autosomal Recessive Congenital Cataract Locus To Chromosome 9q.** *T. Forshew<sup>1</sup>, CA. Johnson<sup>1</sup>, C. Willis<sup>2</sup>, AT. Moore<sup>2</sup>, ER. Maher<sup>1</sup>.* 1) Paediatrics and Child Health, The University of Birmingham, Birmingham, B15 2TG, UK; 2) Institute of Ophthalmology, University College London, England, UK.

Cataracts are a major cause of blindness affecting approximately 16 million individuals worldwide. The genetics of human cataracts are complex, with examples of syndromic and non-syndromic, autosomal dominant, autosomal recessive and X-linked forms described. At least 20 loci for autosomal dominant cataracts have been reported, but only a few non-syndromic recessive loci have been mapped. In order to investigate the molecular basis for recessive cataracts, we investigated two consanguineous families of Pakistani origin. Linkage to candidate genes and loci at 3p, 19q13.4 (*LIM2*), 21q22.3 (*CRYAA*) and the i- blood group locus was excluded (using the criterion of heterozygosity at microsatellite markers) in the largest family. Subsequently we have identified an extensive (20cM) region of homozygosity at 9q21.12-9q22. This region overlaps with the ARPC locus associated with autosomal recessive later-onset pulverulent cataracts. We then demonstrated linkage to 9q in the second family. The maximum cumulative two-point LOD score [ $Z_{\max}$ ] for the two families was 3.378 at  $\theta=0$  for marker D9S303. These findings suggest that congenital and later onset autosomal recessive cataracts may result from allelic heterogeneity at a 9q21.12-q22 locus.

**Candidate regions to Richieri-Costa-Pereira syndrome.** *R.L.L. Ferreira de Lima*<sup>1</sup>, *R. Schultz*<sup>2</sup>, *M. Marazita*<sup>3</sup>, *D. Moretti-Ferreira*<sup>1,4</sup>, *A. Richieri-Costa*<sup>4</sup>, *J.C. Murray*<sup>2</sup>. 1) Departamento de Genética, Universidade Estadual Paulista UNESP, Botucatu, SP, Brazil; 2) University of Iowa, IA, USA; 3) University of Pittsburg; 4) Universidade de São Paulo USP, Bauru, SP, Brazil.

The Richieri-Costa-Pereira syndrome is a rare autosomal recessive disorder characterized by short stature, Robin sequence, cleft mandible, pre/postaxial anomalies and clubfoot (Richieri-Costa and Pereira, 1992). This syndrome was described almost exclusively in Brazil, appearing just one case in French, suggesting this condition represents a rare mutation and founder effects (Richieri-Costa and Pereira, 1993). We have collected fifteen affected from 14 apparently unrelated families with this unique syndrome. In the initial phase of research, DNA from affected individuals were excluded of 497 possible candidate regions and PAX9, MSX1, PITX1, DLX5, DLX6, PITX2, TBX4, TBX5, LIMH1, CLIMB2 and FGF8 as candidate genes (Ferreira de Lima et al., in press). In complementary analysis we typed 387 markers in all families and found evidence for allele sharing in one family with two typed affected. The most positive multipoint LOD results were found under recessive model for chromosomes 9 (LOD=1.49 and TDT p-value=0.08) and 13 (LOD=1.53) given the agreement between the LOD and TDT results, the chromosome 9 regions seems like the most likely to target for the gene. The linkage approaches are done only in the single family and the TDT association approach is done over all families. We will now proceed to high resolution SNP mapping of the two strong candidate regions to look for a block of allele sharing that can focus candidate gene evaluation.

**Linkage analysis using the TSC SNP map.** *B.L. Browning, M.G. Ehm, M.J. Wagner.* Population Genetics, GlaxoSmithKline, Research Triangle Park, NC.

With the recent publication of The SNP Consortium's SNP linkage map there has been increasing attention focused on using clusters of tightly linked single nucleotide polymorphism (SNP) markers for linkage analysis. Although individual SNPs are relatively uninformative, clusters of tightly linked SNPs can be as informative as microsatellite markers. However, SNPs within clusters will typically be in linkage disequilibrium, and the estimates of genetic distances between SNPs in a cluster may exhibit considerable relative error. We evaluated the TSC SNP map to determine the effect of the intracluster LD and intracluster map error on linkage analysis.

We genotyped 83 markers from the TSC linkage map in 3 regions covering 190 cM previously scanned with microsatellite markers and found to be linked to type 2 diabetes (Ehm et al 2000). We found that due to the low intracluster linkage disequilibrium in the TSC SNP map, satisfactory results were obtained from analyzing the TSC markers with existing multipoint linkage analysis software (e.g. GENEHUNTER, MERLIN) provided that the intracluster genetic distances were set to a reasonable small positive distance (e.g. 0.03 cM). We found that the linkage signals were relatively insensitive to changes in estimates of intracluster genetic distances.

22% of the TSC SNP markers in the 3 regions failed to validate on a multiplexed, single base chain extension genotyping platform. For the 83 validated markers, we found the information content and LOD score profiles were similar to a microsatellite map with a 10 cM average spacing. There was comparable evidence for linkage in 2 of 3 regions, and the TSC map yielded weaker evidence for linkage in a region where the TSC map has poor marker coverage.

We recommend that the TSC SNP map be supplemented with additional markers in regions of poor marker coverage, and we suggest that regions showing evidence for linkage be reviewed for possibly error due to intracluster linkage disequilibrium and poorly estimated intracluster genetic distances before proceeding with association studies.

**Replication of a parent of origin effect and genome-wide significant linkage on chromosome 10 for late onset**

**Alzheimer's disease.** *D. Avramopoulos*<sup>1</sup>, *M.D. Fallin*<sup>2</sup>, *R.T. Perry*<sup>3</sup>, *H. Wiener*<sup>3</sup>, *B. Watson*<sup>3</sup>, *R.C.P. Go*<sup>3</sup>, *S.S. Bassett*<sup>1</sup>.

1) Psychiatry, Johns Hopkins University, Baltimore, MD; 2) Epidemiology, Johns Hopkins University, Baltimore, MD; 3) Epidemiology, University of Alabama at Birmingham, Birmingham, AL.

Of all the regions that have been detected in genome scans for late onset Alzheimer's disease (LOAD), only a few have consistently provided positive findings. One such region is on chromosome 10, 60 to 90 cM from the p-telomere. Clinical and epidemiological studies have suggested excess maternal transmission of Alzheimer's disease (AD). Genetic imprinting has been suspected in AD because of parental age effects. Differential chromosome sensitivity to 5-azacytidine in AD has been described suggesting differences in methylation patterns. In a recent paper we reported evidence of linkage in our subset of the NIMH genetics initiative pedigrees, approaching genome-wide significance (non-parametric LOD score = 3.27), when only families with maternal disease origin were analyzed (N=49). This parental origin effect was shown to be statistically significant by multiple random stratifications. We have now acquired parent of origin information for an independent subset of the NIMH genetics initiative pedigrees, those collected by the University of Alabama (UAB). Like in our sample, maternal pedigrees from the UAB set (N=21) show significantly increased linkage in the chromosome 10 region. Combining all maternal pedigrees (N=21+49=70) we acquire a non-parametric LOD score of 4.93 in the same region, which exceeds the conservative threshold for genome wide significant linkage (3.6). These findings add to our previous report in two ways: 1. Replication of the parent of origin effect and 2. Detection of highly significant linkage in a region reported by many investigators. We conclude that this region on chromosome 10 very likely harbors a gene for LOAD, and that mechanisms involving a parent of origin effect, like genomic imprinting, need to be examined.

**Screening validated SNPs for the development of a human genome linkage marker resource.** *K. Doheny<sup>1</sup>, P. Boyce<sup>1</sup>, D. Witmer<sup>1</sup>, D. Hayden<sup>1</sup>, J. Pettengill<sup>1</sup>, Y. Tsai<sup>1</sup>, E. Pugh<sup>1</sup>, E. Luong<sup>2</sup>, R. Nussbaum<sup>2</sup>.* 1) Center for Inherited Disease Research, IGM, JHUSOM, Baltimore, MD; 2) IDR, NHGRI, NIH, Bethesda, MD.

Methods of reducing cost and increasing genotyping throughput are required in order to utilize SNP markers in linkage and association studies. Multiplexing genotyping reactions at both the PCR and detection level is one solution. We are developing a linkage marker resource with the aim of providing quality markers capable of multiplex processing using Sequenom's Homogeneous Mass Extend assay, which discriminates genotypes through MALDI-TOF Mass Spectrometry (Bruker Autoflex). The development process began with a set of 4,524 publicly available SNPs (SNP Consortium, Orchid set) with minor allele frequencies >0.25. Single Base Extension (SBE) assays were designed for these SNPs and multiplexed by their primer, pause, and extension products resulting in 1900 assays grouped into 380 five-plex panels. The assays were screened for utility, 5-plexing at both the PCR and detection level, by genotyping 23 Polymorphism Discovery Resource samples (Coriell). The median genotype call rate was 75%. 965 SNPs were above the median. Genotype reproducibility was tested by typing 50 assays on the 23 samples 16 times each. 8 errors were observed out of 11,664 genotypes (0.07%). Additional results addressing contributing factors to call rates and errors such as multiplex levels and genomic DNA concentration will also be presented. The set of 965 SNPs is distributed across all chromosomes, has an average minor allele frequency of 0.33 and a median inter-marker physical distance of 3.3 Mb. Our reagent costs per genotype using 96 spot chips are currently 5-plex \$0.08, 3-plex \$0.13, 1-plex \$0.40.

**Fine mapping of the apolipoprotein B regulator gene 2 (*Abrg2*) on mouse chromosome 4.** G.P. Ables, B. Reid, A.L. Lin, H. Leung, L.-S. Huang. Medicine, Columbia University, New York, NY.

Elevated plasma levels of apolipoprotein B (apo B) and low density lipoprotein (LDL) are associated with a higher risk for atherosclerotic coronary heart disease (CHD). Using a human apo B transgenic mouse (HuBTg) model, we identified two novel quantitative trait loci (QTL), *Abrg1* and *Abrg2*, with major effects on plasma human apo B levels in crosses between C57BL/6 (B6) and 129/Sv (129). The *Abrg2* is within an interval on chromosome 4 defined by D4Mit27 and D4Mit204 (42.5-61.9 cM) with peak LOD score on D4Mit204 (LOD=8.4) in male mice. Male congenic mice (n=10) heterozygous for the interval (derived from the 129 genome) had significantly lower plasma apo B levels compared to male B6 HuBTg mice (n=193) (769 vs. 9017 mg/dl, p=0.002). Data from subcongenic lines generated between the markers D4Mit164 and D4Mit204 (28.6-61.9 cM) show the low-apo B phenotype. Subcongenic lines generated between D4Mit164 and D4Mit187 (28.6-49.6 cM) had the high-apo B phenotype. These data predict that the *Abrg2* is located in a 12-cM interval between markers D4Mit187 and D4Mit204 (49.6-61.9 cM). However, mean plasma apo B levels of the lines generated within the area of D4Mit164 and D4Mit334 (28.6-57.0cM) were intermediate between levels for B6 HuBTg mice and the other subcongenic lines with a low-apo B phenotype. These data suggest that at least two genes (or gene clusters) are required for a full effect on plasma apo B levels. One of the genes is located between D4Mit187 and D4Mit334 and the other one is located between D4Mit334 and D4Mit204. Our data also showed that the *Abrg2* is located within an interval syntenic to a human chromosome segment associated with a QTL for familial combined hyperlipidemia (FCHL), a very prevalent disorder in general populations. Overall, our data suggest that the *Abrg2* QTL consists of more than one gene or gene clusters and it is strong candidate gene for a human FCHL locus.

**Fine Mapping on Chromosome 6q in the NIMH Genetics Initiative Bipolar Pedigrees.** *S. Buervenich<sup>1</sup>, S.D. Detera-Wadleigh<sup>1</sup>, N. Akula<sup>1</sup>, C.J.M. Thomas<sup>1</sup>, L. Kassem<sup>1</sup>, A. Rezvani<sup>1</sup>, W.R. Corona<sup>1</sup>, W. Berrettini<sup>2</sup>, W. Byerley<sup>2</sup>, W. Coryell<sup>2</sup>, J.R. DePaulo Jr<sup>2</sup>, E.S. Gershon<sup>2</sup>, J. Kelsoe<sup>2</sup>, M.G. McInnis<sup>2</sup>, D.L. Murphy<sup>2</sup>, J.I. Nurnberger Jr<sup>2</sup>, T. Reich<sup>2</sup>, W. Scheftner<sup>2</sup>, F.J. McMahon<sup>1</sup>.* 1) National Institute of Mental Health Intramural Research Program, Bethesda, MD; 2) The NIMH Genetics Initiative for Bipolar Disorder (Coordinating Center: Indiana University School of Medicine, Indianapolis, IN).

Whole-genome scans in 2 successive collections of NIMH Genetics Initiative bipolar disorder pedigrees have led to identification of two linkage signals on the long arm of chromosome 6. Both sets of samples were collected using the same ascertainment criteria and diagnostic tools. However, the first signal peaked at marker D6S311 (6q24.3), while in the second sample the strongest linkage was at D6S1021 (6q16.3). Based on these results, we have selected 31 markers from the Decode map, with a 3cM average spacing on 6q, for genotyping in the complete NIMH bipolar disorder pedigree series (n=2000). With about 70% of the genotypes finished, we have so far not observed any substantial change in the magnitude or resolution of the linkage signals on 6q. However, alleles of 2 markers near positional candidate genes on 6q have shown preliminary evidence of association with bipolar disorder in both pedigree samples by the family-based association test (FBAT): D6S1642, located within the GRIK2 gene that encodes a kainic acid-type glutamate receptor, and D6S1009, located close to the interleukin-20 receptor alpha gene (IL20RA) at the intersection of the 2 linkage peaks. There is also some evidence that the associated alleles of D6S1009 partition the linkage evidence on 6q in the NIMH3 sample: the 90 families harboring at least one of the associated alleles produced a significantly higher non-parametric lod-score than the 160 families that did not carry any associated alleles (1.73 vs. 0.573, p=0.02). Based on these findings, IL20RA and GRIK2 are considered high-priority candidate genes for association studies using dense sets of SNPs, alongside other positional candidates PREP, NR2E1, FOXO3A, and APOGL5, which have been prioritized for study on neurobiological grounds.

**Fine-mapping and further localization of schizophrenia susceptibility loci from a genome-wide linkage scan among Ashkenazi Jewish families.** *M. Fallin*<sup>1</sup>, *V.K. Lasseter*<sup>2</sup>, *P.S. Wolynec*<sup>2</sup>, *J.A. McGrath*<sup>2</sup>, *G. Nestadt*<sup>2</sup>, *D. Valle*<sup>3,4,5</sup>, *KY. Liang*<sup>6</sup>, *A.E. Pulver*<sup>2</sup>. 1) Dept Epidemiology, Johns Hopkins School of Public Health, Baltimore, MD; 2) Department of Psychiatry & Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD; 3) Departments of Pediatrics and Molecular Biology, Johns Hopkins School of Medicine, Baltimore, MD; 4) Institute for Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, MD; 5) Howard Hughes Medical Institute; 6) Dept Biostatistics, Johns Hopkins School of Public Health, Baltimore, MD.

Previous linkage studies in schizophrenia have been discouraging due to inconsistent findings and weak signals. Genetic heterogeneity has been cited as one of the primary culprits for such inconsistencies. We have performed a 10cM autosomal genome-wide linkage scan for schizophrenia susceptibility regions using 29 multiplex families of Ashkenazi Jewish descent, in hopes of reducing genetic heterogeneity among families and increasing the detectable effects of any particular locus. We pursued both allele-sharing and parametric linkage analyses as implemented in Genehunter V2.0. We found 7 regions with NPL scores greater than 2.00 (chromosomes 1p32.2, 4q34.3, 6p21.31, 7p15.2, 10q22.3, 15q11.2 and 21q21.2). Our strongest signal was achieved on chromosome 10, with a nonparametric linkage score (NPL) of 3.35 (genome-wide empirical p-value=0.035) and a dominant heterogeneity lod score (HLOD) of 3.14. We have now followed up the regions on chromosomes 6, 10, and 21 to achieve a 1Mb density, and have increased marker content on chromosomes 4, 7, and 15 as well. We present results of fine-mapping for each of these 6 chromosomes, highlighting our encouraging findings on chromosome 10, where the peak NPL score increased to 4.27 (D10S1774; empirical p-value = 0.00002), and a 95% confidence interval of 12.2 Mb for the location of the trait locus was delineated (D10S1677 to D10S1753).

**A genome scan of specific language impairment loci in families from the United States.** *C.W. Bartlett<sup>1</sup>, J.F. Flax<sup>2</sup>, W. Li<sup>1</sup>, T. Reaple-Bonilla<sup>2</sup>, J. Hayter<sup>1</sup>, L.S. Hirsch<sup>2</sup>, M.W. Logue<sup>3</sup>, R. Zimmerman<sup>1</sup>, V.J. Vieland<sup>3</sup>, P. Tallal<sup>2</sup>, L.M. Brzustowicz<sup>1,4</sup>.* 1) Dept Genetics, Rutgers Univ, Piscataway, NJ; 2) Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ; 3) Dept of Biostatistics, Division of Statistical Genetics, College of Public Health and Dept of Psychiatry, College of Medicine, University of Iowa, Iowa City; 4) Dept of Psychiatry, UMDNJ, Robert Wood Johnson Medical School, Piscataway.

Specific language impairment (SLI), affecting ~ 7% of children entering school, is defined as a failure to develop language normally in the absence of other explanatory factors such as hearing loss, autism or mental retardation. Children with SLI typically have difficulty on a wide range of phonologically based reading and language tasks, and many later develop dyslexia (DYX). We have conducted a genome-scan for language impairment susceptibility genes on 22 multiplex nuclear and extended pedigrees ascertained in the United States. Families were analyzed using three diagnostic categories: language impairment (language score > 1 SD below population mean), reading impairment (single nonword reading > 1 SD below nonverbal IQ) and clinical impairment (residual language difficulties defined as either language or reading difficulties). Microsatellite markers were genotyped at ~10 cM resolution. Two-point linkage analysis using MLINK assuming reduced penetrance under both dominant and recessive modes of inheritance was performed. For the clinical impairment diagnosis, HLOD scores > 2 were obtained on 18p11 (dominant MOI) and 1q31 (recessive). The peak on 18p11 is ~30 cM away from the peak of the DYX6 locus. The reading impairment diagnosis yielded HLOD scores > 2 on 7q11 (dominant) and 6p21 (recessive). The result on 6p21 is within the DYX2 region. Previous genome scans for SLI susceptibility indicated loci on 13q, 16q and 19q (Bartlett et al 2002, SLIC 2002). This sample produced HLOD scores > 1 on both 13q and 19q. The locus on 19q was positive for multiple traits and models, perhaps indicating pleiotropy. Our results indicate a genetic overlap of SLI and dyslexia on 6p21, show several loci are unique to SLI, and may replicate linkage of SLI to 13q and 19q.

**Examination of multilocus association of the GABAA receptor subunit genes in multiplex autism families.** *A.E. Ashley-Koch, E.R. Martin, J. Jaworski, M.M. Menold, C.A. Haynes, M.L. Cuccaro, J.R. Gilbert, M.A. Pericak-Vance.*  
Duke University Medical Center, Durham, NC.

We have previously shown both linkage and association to the GABA receptor subunit GABRB3 on chromosome 15q11-q13 in autism. Given that the GABAA receptor genes, GABRA5 and GABRG3, are located in a cluster with GABRB3 on 15q11-q13 and are functionally related to GABRB3, it is possible that an intergenic combination, or combinations of alleles in this family of genes may influence susceptibility to autism. We examined 210 multiplex families with autism (including 99 from the Autism Genetic Exchange) using a novel statistical method, the multilocus geno-PDT, to detect multilocus associations in these three genes. The two SNPs genotyped in GABRB3 were located in intron 6 and the 3UTR. For GABRA5, three SNPs were genotyped in exon 8 and introns 7 and 9. For GABRG3, two SNPs were genotyped in exon 8 and the 5UTR. Global p-values and p-values for individual genotype combinations were computed. Significant association was detected between GABRA5 exon 8 and GABRG3 5UTR (global  $p=0.04$ , max indiv  $p=0.02$ ). Marginal results were found for GABRA5 intron 9 and GABRG3 exon 8 (global  $p=0.08$ , max indiv  $p=0.01$ ), GABRA5 intron 9 and GABRG3 5UTR (global  $p=0.09$ , max indiv  $p=0.04$ ), as well as GABRB3 intron 6 and GABRG3 5UTR (global  $p=0.09$ , max indiv  $p=0.01$ ). Most of these multilocus associations were explained by significant single locus geno-PDT results. However the GABRA5 intron 9/GABRG3 exon 8 association was not detected by single locus results and may represent gene-gene interactions between these loci in autism etiology. We are currently genotyping our sporadic autism families for these SNPs to further examine the possibility of multilocus associations within the GABAA receptor genes clustered on chromosome 15 as a mechanism for autism etiology.

**Linkage Analysis of C-peptide Concentrations in a Cohort of Siblings with Type 2 Diabetes from West Africa.** *G. Chen, J. Zhou, H. Daniel, Y. Chen, C. Rotimi.* Natl Human Genome Ctr, Howard Univ, Washington, DC.

C-peptide is a substance that the pancreas releases into the circulation in equimolar amounts to insulin. It has been considered to be biologically inactive, general view that C-peptide serves a spacer during the biosynthesis of insulin to promote efficient translocation and folding of pro-insulin. Recently, C-peptide has been demonstrated important physiological effects of the C-peptide itself which relate to the vascular field, in particular to the microcirculation. For log transformation of C-peptide, additive genetic heritability is 0.79 (0.11) ( $P < 0.0000001$ ), Kurtosis is 0.39 in our African American Diabetes Mellitus (AADM) study. To identify genes contributing to C-peptide variation, we conducted a genome wide scan in an average 9cM genome map of 373 type-2 diabetes affective sib-pairs in 343 pedigrees (10.52% involved the treatment of insulin). Using variance components approach in SOLAR package, we obtained LOD Score = 3.42 in chromosome 18 (7cM, between markers C18S1781 and D18S976), a LOD Score = 3.10 (33cM, D4S2639) in chromosome 4, a LOD Score = 1.41 in chromosome 10 (107cM, between markers D10S2327 and D10S2470) and a LOD = 2.20 in chromosome 15 (43cM, D15S659). In conclusion, since C-peptide is along with insulin in bloodstream and has some amount as insulin, the finding of linkage regions for C-peptide is as same as finding of linkage regions for insulin and also studies have been demonstrated physiological effects of C-peptide involving renal and never function in diabetes. Our findings provided the significant linkage evidences in 18p11 and 4p15 regions and suggestive linkage evidences in 15q12 and 10q22 in a cohort of siblings with type 2 diabetes from West Africa.

**Reduction in the minimal candidate region of cerebral cavernous malformations type 3 (CCM3) on chromosome 3q26.31-27.3.** C.L. Liquori<sup>1</sup>, V. Maglione<sup>2</sup>, F. Balogun<sup>1</sup>, M. Cannella<sup>2</sup>, E. Huang<sup>1</sup>, L. Hughes<sup>1</sup>, T. Leedom<sup>1</sup>, F. Squitieri<sup>2</sup>, D.A. Marchuk<sup>1</sup>. 1) Dept. Mol. Genet. and Microbiol., Duke University, Durham, NC; 2) Dept. Mol. Pathology, Mediterranean Neurological Institute, Pozzilli, Italy.

Cerebral cavernous malformations (CCMs) are vascular abnormalities of the brain that can result in a variety of neurological disabilities, including hemorrhagic stroke and seizures. CCMs can be sporadic or inherited in an autosomal dominant fashion. There are three known loci that are responsible for familial CCM. The gene responsible for CCM1 is *KRIT1*. The genes responsible for CCM2 and CCM3 have not yet been identified, and their loci map to chromosome bands 7p15-13 and 3q25.2-27, respectively. In order to identify potential CCM2 and CCM3 families, we have collected CCM families and have excluded those with mutations within *KRIT1* by sequence analysis. We ran a panel of markers for both the CCM2 and CCM3 loci on these families, including the previously published family CAV02 (Squitieri, *et al.*, *Neurol Sci* 21:129-34.). In the CAV02 family, the affected parent passed on one haplotype to both affected children and the other haplotype to the unaffected (MRI negative, 3 times over 8 years) child for the CCM3 region, indicating that the CAV02 family has CCM3. In addition, we identified a recombination event in one of the affected siblings. By designing several new polymorphic markers, we were able to define the site of recombination to a 55.2 kb region. Previously published studies (Craig, *et al.*, *Hum Mol Genet* 7: 1851-8.) only established lod-1 and lod-3 support intervals for both the CCM2 and CCM3 loci. The CAV02 recombination event establishes a definitive centromeric border for the CCM3 locus. It also reduces the size of the critical region by 1.5 Mb (based on using the markers defining the lod-1 support interval to arbitrarily define the CCM3 locus boundaries). This recombination event also excludes 7 genes from consideration as candidate genes. In addition, we have sequenced 13 candidate genes and did not identify any mutations in these genes. By reducing the size of the critical region and excluding several candidate genes, we have made progress towards the identification of the CCM3 gene.

**A genome-wide screen for myopia susceptibility loci in twins, including a replicate sample.** *C.J. Hammond, T. Andrew, M.J. Barber, T.D. Spector.* Twin Research & Genetic Epidemiology Unit, St Thomas' Hospital, London, UK.

**Purpose:** Myopia has been shown to be under strong genetic control with a heritability based on twin studies of around 85%. Susceptibility loci have been identified in families with AD high myopia. The purpose of this genetic epidemiological study was to identify regions of the genome that contain quantitative trait loci (QTL) for refractive error, as no loci have been identified to date in population samples.

**Methods:** Refractive error in 221 unselected female dizygotic twin pairs from the St Thomas' Adult Twin Registry was obtained using a Humphrey 670 autorefractor, and the spherical equivalent obtained for each sib-pair. A genome-wide screen using around 400 markers was performed. Non-parametric multipoint linkage analyses were undertaken, using optimal Haseman-Elston methods implemented in a generalised linear model (gamma regression) and using the square of sib phenotypic differences. A replicate sample of different twins, whose refractive error was obtained by postal questionnaire, was similarly analysed.

**Results:** Maximum evidence of linkage in the autorefracted twins was observed at chromosome 11p13 (LOD 6.1), with further loci at chromosomes 3q26 (LOD 3.7) and 4q12 (LOD 3.3) and 8p23 (LOD 4.1). Evidence for these loci remained or became stronger after checking model fit diagnostics and removing unduly influential outliers. Weaker evidence of linkage ( $1.0 < \text{LOD} < 3.0$ ) was also obtained for several other regions. Possible replication was produced at the 3q26 locus for 170 twin pairs returning questionnaires. Additional twins are being recruited, to increase the power to replicate these results.

**Conclusions:** These results, if confirmed in our ongoing replicate sample and in other studies, provide strong evidence of possible susceptibility loci for refractive error, and warrant characterization of QTLs and candidates responsible.

**Linkage Analysis in Families with Joubert Syndrome plus Oculo-Renal Involvement Identifies the CORS2 Locus on Chromosome 11p12-q13.3.** *L.C. Keeler<sup>1</sup>, S.E. Marsh<sup>1</sup>, C.G. Woods<sup>2</sup>, A. Gururaj<sup>3</sup>, L. Al-Gazali<sup>3</sup>, L. Sztriha<sup>3</sup>, J.G. Gleeson<sup>1</sup>.* 1) Neurogenetics Laboratory Division of Pediatric Neurology Department of Neurosciences University of California, San Diego, CA ; 2) Molecular Medicine Unit and Yorkshire Clinical Genetics Service St James's University Hospital Leeds, UK; 3) Department of Pediatrics Faculty of Medicine and Health Sciences United Emirates University Al Ain, United Arab Emirates.

Joubert syndrome (JS) is an autosomal recessive developmental brain condition characterized by hypoplasia/dysplasia of the cerebellar vermis and by ataxia, hypotonia, oculomotor apraxia, and neonatal breathing dysregulation. A form of JS that includes retinal dysplasia and cystic dysplastic kidneys has been differentiated from other forms of JS, called either JS type B or Cerebello-Oculo-Renal syndrome (CORS), but the genetic basis of this condition is unknown. Here we describe three consanguineous families that display CORS. Linkage analysis defines a novel locus on chromosome 11p12-q13.3, with a maximum two-point LOD score of  $Z = +5.2$  at the marker D11S1915. Therefore, the cerebello-oculo-renal form of JS is a distinct genetic entity from the previously described Joubert Syndrome 1 (JBTS1) locus, in which there is minimal involvement of retina or kidney. We suggest the term CORS2 for this new locus. Several additional families fail to map to either JBTS1 or CORS2, indicating further genetic heterogeneity.

**Autosomal dominant Retinitis Pigmentosa: evidence for a novel disease-causing gene.** *L. Jiang<sup>1, 2</sup>, M. Payne<sup>1, 2</sup>, N. Faulkner<sup>1, 2</sup>, M. Machan<sup>1, 2</sup>, S. Sims<sup>1, 2</sup>, K. Hart<sup>1, 2</sup>, X. Li<sup>1, 2</sup>, P. Bernstein<sup>1, 2</sup>, Z. Yang<sup>1, 2</sup>, K. Zhang<sup>1, 2</sup>.* 1) Department of Ophthalmology and Visual Science, University of Utah Health Science Center, Salt Lake City, UT80132; 2) Programme in Human Molecular Biology and Genetics, University of Utah Health Science Center, Salt Lake City, UT80132.

**Purpose:** Retinitis pigmentosa (RP) is a type of inherited retinal degenerations characterized by progressive night blindness and loss of peripheral vision, with eventual loss of central vision. RP is the most prevalent group of inherited retinopathies, affecting approximately 1 in 3,500 persons. RP is genetically heterogeneous and may present as an adRP, autosomal recessive, or X-linked recessive trait. To date, at least 13 autosomal dominant genes/loci have been localized and 12 genes have been identified (RetNet [www.sph.uth.tmc.edu/RetNet/](http://www.sph.uth.tmc.edu/RetNet/)). Loci include RP1, RP4(Rhodopsin), RP7(RDS/Peripherin), RP18(HPRP3), RP9(PIM1K), RP10(IMPDH1), RP11(PRP31), RP13(PRPC8), RP17, RP27(NRL), ROM1, Fascin, CRX. **Methods:** A large Mormon kindred with a total of 144 members (20 members affected by RP) was studied. Of 46 individuals who participated in the study, 12 were found to be affected. The disease gene is transmitted as an autosomal dominant trait. Linkage analysis to all known adRP loci and mutational screening of PIM1K, RDS/Peripherin, NRL, CRX, ROM1, and Fascin were performed. **Results:** Affected individuals showed night blindness, and constriction of peripheral visual fields, with loss of central vision in the advanced stage of the disease. Fundus examinations revealed retinal and RPE atrophy, bone spicule pigmentation, arterial attenuation in affected individuals. Linkage analysis using short-tandem repeat polymorphic markers encompassing candidate loci showed that none of above described loci was linked to the phenotype in this kindred. Direct sequencing of RDS/peripherin, RP9, NRL, CRX, ROM1 and Fascin showed no mutation in any of the affected individuals. **Conclusions:** We identified a large Mormon family with an autosomal dominant RP. Linkage analysis and candidate gene sequencing have excluded known genes. A genome-wide scan is underway to identify a potentially novel RP locus in this kindred.

**A genome scan for modifiers of age at onset in Huntington's disease: The HD MAPS study.** *J.L. Li<sup>1,2</sup>, M. Hayden<sup>3</sup>, E.W. Almqvist<sup>3</sup>, A. Durr<sup>3</sup>, C. Dod<sup>3</sup>, P.J. Morrison<sup>3</sup>, O. Suchowersky<sup>3</sup>, C.A. Ross<sup>3</sup>, R.L. Margolis<sup>3</sup>, E. Gmez-Tortosa<sup>3</sup>, D. Mayo<sup>3</sup>, A. Novelletto<sup>3</sup>, M. Nance<sup>3</sup>, R.J.A. Trent<sup>3</sup>, A. Zanko<sup>3</sup>, R.K. Abramson<sup>3</sup>, A.L. Russ<sup>1</sup>, J.F. Gusella<sup>4</sup>, M.E. MacDonald<sup>4</sup>, R.H. Myers<sup>1,2</sup>.* 1) Neurology, Boston University School of Medicine, Boston, MA; 2) Bioinformatics Program, Boston University, Boston, MA; 3) The HD MAPS Study Group; 4) Molecular Neurogenetics Unit, Massachusetts General Hospital, Boston, MA.

Huntington's disease (HD) is caused by the expansion of a CAG repeat within the coding region of a novel gene on 4p16.3. While the variation in age at onset is partly explained by the size of the expanded repeat, the remaining variation in onset age is strongly heritable ( $h^2 = 0.56$ ) suggesting that other genes modify the onset age of HD. To identify these modifier loci, we performed a 10-cM density genome-wide scan in 629 affected sibling pairs (295 pedigrees and 695 individuals). Ages at onset were adjusted by linear regression for the effects of the expanded CAG repeat length, the normal repeat size and an interaction term of these two factors, and standardized residuals were generated for linkage analysis. The obtained residuals were normally distributed. Because all those studied were HD affected, estimates of allele sharing identical by descent at and around the HD locus were adjusted by a positionally weighted method to correct for the increased allele sharing at 4p. Suggestive evidence for linkage was found at 4p16 (LOD=1.93), 6p21-23 (LOD=2.29), and 6q24-26 (LOD=2.28). These regions may be useful for investigation of genes that modify age at onset of HD.

**Genome-wide scan of obesity-related traits in Mexican American coronary artery disease families.** *X. Li<sup>1</sup>, M.J. Quiñones<sup>2</sup>, D. Wang<sup>1</sup>, I. Enriquez<sup>2</sup>, X. Jimenez<sup>2</sup>, G. Hernandez<sup>2</sup>, R. De La Rosa<sup>2</sup>, W.A. Hsueh<sup>2</sup>, J.I. Rotter<sup>1</sup>, H. Yang<sup>1</sup>.*  
1) Cedars-Sinai Medical Center, Los Angeles, CA; 2) UCLA, Los Angeles, CA.

Obesity is an independent risk factor for coronary artery disease (CAD). To identify the genetic determinants of obesity, we performed a genome-wide linkage analysis of obesity-related traits using data from 101 Mexican American nuclear families ascertained via a parent (proband) with documented CAD. The available obesity traits include body mass index (BMI), body surface area (BSA), waist-to-hip ratio (WHR), fat mass as percent of body weight (FM%), and percent truncal fat (FM-T%). These traits were measured in adult offspring and offspring spouses (n=438). 568 adult offspring and their parents were genotyped for 408 microsatellite markers along the genome at ~10cM density. Heritability estimates and multipoint linkage analysis was performed using a variance components procedure implemented in SOLAR. After taking into account age and gender effects, the heritability estimates were 0.62 for BMI, 0.73 for BSA, 0.40 for WHR, 0.39 for FM% and 0.38 for FM-T% (all  $P < 0.001$ ). Evidence for linkage was observed at 252~267cM on chromosome 2 for four obesity-related traits (except for WHR). The maximum LOD scores (LOD) were 2.35 for BMI, 3.16 for BSA, 2.80 for FM% and 2.62 for FM-T%. We also observed suggestive linkage for BMI and BSA on chromosomes 5 (BMI: LOD = 2.18 at 167 cM; BSA: LOD=2.06 at 163 cM). Additional linkage signals were observed for BMI on chromosome 4 (LOD = 1.42 at 204 cM), for BSA on chromosome 8 (LOD=2.25 at 119 cM), for FM-T% on chromosome 11 (LOD=1.41 at 123 cM) and chromosome 16 (LOD = 1.48 at 51 cM). These results indicate that 1) there is a strong genetic influence on obesity in CAD families, and 2) the greatest evidence for linkage is observed for a group of obesity-related traits at approximately the same locus on chromosome 2q.

**A genome wide search for Kawasaki Disease susceptibility genes.** *Y. Onouchi<sup>1</sup>, S. Onoue<sup>1</sup>, M. Tamari<sup>2</sup>, K. Wakui<sup>3</sup>, Y. Fukushima<sup>3</sup>, M. Yashiro<sup>4</sup>, Y. Nakamura<sup>4</sup>, H. Yanagawa<sup>5</sup>, T. Kawasaki<sup>6</sup>, Y. Nakamura<sup>7,8</sup>, A. Hata<sup>1,9</sup>.* 1) Lab Gastrointestinal Diseases; 2) Lab. Genetics of Allergic Diseases, SNP Research Center RIKEN, Yokohama, Japan; 3) Dept. Preventive Medicine, Shinshu Univ. School of Medicine, Matsumoto, Japan; 4) Dept Public Health, Jichi Medical School, Tochigi, Japan; 5) Saitama Prefectural Univ, Koshigaya, Japan; 6) Japan Kawasaki Disease Research Center, Tokyo, Japan; 7) Lab. Molecular Medicine, Human Genome Center, Inst. Medical Science, the University of Tokyo, Tokyo, Japan; 8) Research Group for Personalized Medicine, SNP Research Center, RIKEN, Yokohama, Japan; 9) Dept. Public Health, Graduate School of Medicine, Chiba Univ, Chiba, Japan.

Kawasaki disease (KD), mucocutaneous lymphnode syndrome (MCLS), is an acute systemic vasculitis syndrome of young children. Although initially thought to be a benign illness, it is now known that coronary artery abnormalities develop in approximately 15 to 25% of children with untreated KD. KD is a leading cause of acquired heart diseases of children in the developed countries. Based on clinical and epidemiological features, it had been thought that some infectious agents trigger the activation of immune system and cause the KD phenotype, but the etiology remains unknown. The high incidence of KD in the population of Japanese ancestry, high relative recurrent risk for siblings ( $s=10$ ) and male predominacy suggest that there exist genetic factors. Here we performed genome wide search using 81 Japanese affected sib pairs to identify genes determining susceptibility for KD. 399 microsatellite markers were genotyped for these sib pairs, if available, their parents and unaffected siblings. Multipoint linkage analysis using MAPMAKER/SIBS program, and single point analysis using SIBPAL program was conducted. 8 chromosomal region showed supportive evidence of linkage of  $MLS=1.0$  or higher. In 12q24, 7p15, 19q13.3, relatively high LOD score ( $MLS=3.1, 2.9, 2.2$  respectively) was observed after additive markers were analyzed. It was suggested that major susceptibility genes for KD lie within these regions and now case control association study with SNPs in the candidate genes is in progress.

**Dense genome scan for rheumatoid arthritis (RA).** *J. Osorio y Fortea*<sup>1</sup>, *H. Bukulmez*<sup>2</sup>, *E. Petit*<sup>1</sup>, *L. Michou*<sup>1</sup>, *C. Pierlot*<sup>1</sup>, *S. Cailleau*<sup>1</sup>, *S. Lasbleiz*<sup>3</sup>, *T. Bardin*<sup>4</sup>, *B. Prum*<sup>3</sup>, *J. Olson*<sup>2</sup>, *F. Cornelis*<sup>1,2</sup>. 1) GenHotel/Laboratoire de recherche Europeen pour la polyarthrite rhumatoide, Universite d'Evry, Evry, France; 2) Department of epidemiology and biostatistics, Case Western Reserve University, Cleveland, Ohio; 3) Unite genetique clinique, hopital Lariboisiere, Paris, France; 4) Laboratoire statistique et genome, genopole, tour Evry2, Evry, France.

**Background** RA is the most frequent autoimmune disorder (1% prevalence). It is a complex disease ( $s = 2 - 10$ ), with HLA locus accounting for 1/3 of the familiarity. The European Consortium on RA Families reported in 1998 a 12 cM scan, suggesting 26 non-HLA loci ( $P < 0.05$ )

**Aim** Produce a 4 cM scan and 10000 simulations to refine the analysis

**Methods** 88 French affected sibling pair families were studied with 1088 markers. Linkage analysis was performed with Allegro1.1. Assessment of the statistical significance was performed with 10000 simulations

**Results** The average marker density was 3.3 cM. HLA linkage was found ( $P = 6.2 \cdot 10^{-5}$ ) and 19 regions were suggested at the 5% significance level, on chromosomes 1 to 6, 12, 13, 16, 18, 20, 22 and X. The probability of observing  $\geq 19$  non-HLA hits by chance was  $P < 0.05$ , suggesting the existence of RA loci. The average number of false positives was 11, suggesting 8 true linkages (19 observed - 11 expected). From the 26 regions suggested in 1998, only 9 remained and 11 were new. No region was found to overlap with all RA scans reported, by the Japanese, North American and English groups, presumably for linkage power and marker density issues. Some regions overlapped with those found for other autoimmune diseases and animal model scans, suggesting the existence of shared autoimmune genes

**Conclusion** We report a 3.3 cM genome scan for RA, suggesting the existence of non-HLA RA loci ( $P < 0.05$ ). Such a dense genome scan permitted to refine the description of candidate RA loci, some of which might be autoimmune loci. Simulations were consistent with an estimation of 8 genuine RA loci.

**Genome scan of cleft lip with or without cleft palate (CL/P): Part II: Broadening the phenotype to include velopharyngeal incompetence (VPI).** *K. Neiswanger<sup>1</sup>, M.D. Ford<sup>2</sup>, M.E. Cooper<sup>1</sup>, T.H. Goldstein<sup>1</sup>, S.S. Petiprin<sup>1</sup>, K.M. Bardi<sup>1</sup>, C.A. Brandon<sup>1</sup>, R.F. Giles<sup>1</sup>, M.L. Marazita<sup>1</sup>.* 1) Center for Craniofacial and Dental Genetics, Univ Pittsburgh Sch Dent Med, Pittsburgh, PA; 2) Cleft Palate-Craniofacial Center, Univ Pittsburgh Sch Dent Med, Pittsburgh, PA.

Nonsyndromic CL/P is a common birth defect with a complex, multifactorial etiology. Individuals with CL/P have a variable range of speech deficits, including anatomical and/or functional problems with their velopharyngeal (VP) mechanism. The purpose of this study is to seek genetic regions that may be important in the VP portion of the CL/P phenotype. We used the University of Pittsburgh Weighted Values for Speech Symptoms Associated with VPI scale to assess the VP status of 36 CL/P and 86 unaffected family members in 23 multiplex, nonsyndromic CL/P Caucasian families ascertained through the University of Pittsburgh Cleft Palate-Craniofacial Center. Scores of 0 on this assessment are considered VP competent (unaffected), and scores of 1 or higher are considered VPI (affected). We performed a genome scan using 392 anonymous markers spaced an average of 9 cM apart (from CIDR). Single-point and multipoint parametric and nonparametric linkage analyses were performed. We calculated the results twice--first using CL/P as the affected phenotype, and then repeating the analysis with a broad definition that added 19 non-CL/P family members with VPI scores > 0. The highest single-point LOD scores using the broad definition of affection status were +1.95, +1.74, and +1.74 for markers on chromosomes 5, 1 and 7, respectively. None of these scores occurred within 30 cM of the highest LOD scores on each chromosome under the narrow definition. These results suggest that the genes predisposing to VPI are not the same set of genes as the primary CL/P genes. Instead, they may modify the expression of primary CL/P genes, or act to alter the VP mechanism independently of clefting. Supported by NIH grants DE13076 & RR00084; genotyping grant from the Center for Inherited Disease Research.

**Significant linkage of attention-deficit/hyperactivity disorder (ADHD) to three genomic regions: 5p13, 6p12, and 17p11.** *M.N. Ogdie<sup>1</sup>, S.E. Fisher<sup>4</sup>, S.K. Loo<sup>2,3</sup>, M. Yang<sup>2</sup>, J.J. McGough<sup>3</sup>, J.T. McCracken<sup>3</sup>, A.P. Monaco<sup>4</sup>, S.L. Smalley<sup>2,3</sup>, S.F. Nelson<sup>1,2,3</sup>.* 1) Dept Human Genetics, UCLA, Los Angeles, CA; 2) Center for Neurobehavioral Genetics, UCLA, Los Angeles, CA; 3) Department of Psychiatry and Biobehavioral Sciences, UCLA, Los Angeles, CA; 4) Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, UK.

A previous genome-wide scan in 270 ADHD affected sibling pairs (ASPs) identified 5 novel independent regions of linkage (Ogdie et al. 2003). We have fine-mapped all 5 candidate regions in an extended population sample comprising 230 multiplex families and 306 ASPs. Parents and siblings were genotyped at 2cM density and maximum-LOD score (MLS) analysis was performed in Genehunter v. 2.1. MLS analysis yielded three regions with significant linkage to a broad definition of ADHD: 5p13 (MLS 3.60,  $P = 2.3 \times 10^{-5}$ ), 6p12 (MLS 3.93,  $P = 1.0 \times 10^{-5}$ ), and 17p11 (MLS 3.62,  $P = 2.2 \times 10^{-5}$ ). Simulations within the specific parameters of this data set indicate that all three regions present MLS values above the threshold for significance. 10,000 replicates of each chromosome under the null hypothesis produced the following approximations of the empirical p-values: 5p13 ( $P = 0.0016$ ), 6p12 ( $P = 0.0012$ ), and 17p11 ( $P = 0.0016$ ). Estimates for sibling relative risk ratios (s), derived from sharing parameters, indicate s values of approximately 1.4 to 1.5 for all three regions. Thus, all three genomic intervals constitute candidate regions for susceptibility loci of moderate effect size.

**A genome-wide search for angiotensin-I converting enzyme (ACE) quantitative trait loci in Nigerian families.**

*C.A. McKenzie<sup>1</sup>, A. Adeyemo<sup>2</sup>, X. Zhu<sup>3</sup>, N. Bouzekri<sup>4</sup>, T. Forrester<sup>1</sup>, R.S. Cooper<sup>3</sup>.* 1) Tropical Metabolism Research Unit, University of the West Indies, Kingston, Jamaica; 2) Dept. of Pediatrics/Institute for Child Health, University of Ibadan, Ibadan, Nigeria; 3) Dept. of Preventive Medicine and Epidemiology, Loyola University Medical Center, Maywood, IL; 4) Institute of Biological Anthropology, University of Oxford, Oxford, UK.

Angiotensin I-converting enzyme (ACE) appears to play a critical role in the maintenance of cardiovascular homeostasis. There is well-characterised evidence from measured haplotype and other analyses conducted in different ethnic groups that circulating ACE levels are influenced by one or more quantitative trait loci (QTLs) either within or near to the ACE gene on chromosome 17. In several of these studies there are residual familial correlations, after accounting for the ACE-linked QTL, which are compatible with the presence of unlinked genetic factor(s) - no such loci have been identified as yet. The high heritability of circulating ACE suggests that the prospects for mapping ACE QTLs may be evaluated in samples of relatively modest size.

As part of a larger study of hypertension in the African diaspora, a genome-wide panel of microsatellite markers has been typed in 2 Nigerian family panels in which circulating ACE levels were also available. In a subset of these families single nucleotide polymorphisms (SNPs) in the ACE gene were also typed. In an effort to map putative QTL(s) unlinked to the ACE gene, we have analysed these marker and phenotype data using FASTLINK. Single locus models with fixed parameters were used to compute lod scores for linkage to ACE; several markers in both family sets showed modest evidence of linkage (i.e. lod scores > 1.5) including a marker on chromosome 17 with a lod score > 3.0. We also explore analyses of linkage to ACE levels and to ACE levels adjusted for the effects of ACE gene markers by computing lod scores with fixed parameters or by maximising the likelihood over theta and the QTL parameters joint.

Program Nr: 1898 from 2003 ASHG Annual Meeting

**A comprehensive human genetic map using all currently available CEPH markers.** *M. Jirout, C.C. Abney, N.J. Schork.* Dept of Psychiatry, UCSD School of Medicine, La Jolla, CA.

We have developed a comprehensive genetic map using all available marker data in the CEPH repository. This map consists of over 14,000 microsatellite, SNP, RFLP and in/del variations. We compared our new recombination frequency data to available physical map information. In addition, we have compared recombination frequencies obtained from the CEPH data to recombination rates observed in syntenic regions in the mouse and rat genomes.

**Parkinsonism Linked to Chromosome 2 and 18 in an Extended Amish Family.** *S. Lee*<sup>1</sup>, *Y. Bradford*<sup>2</sup>, *A. Crunk*<sup>2</sup>, *E. Bratton*<sup>1</sup>, *M. Allen*<sup>2</sup>, *J. Haines*<sup>2</sup>. 1) Dept Neurology, Vanderbilt Univ, Nashville, TN; 2) Dept. Molecular Physiology and Biophysics, Program in Human Genetics, Vanderbilt Univ, Nashville, TN.

Causative gene mutations identified in familial Parkinson disease (PD) have yielded insights into the pathogenesis of this neurodegenerative disorder. We previously reported an Amish population in Ohio with parkinsonism and dementia whose clinical features include typical idiopathic PD as well as progressive supranuclear palsy. Given the complex nature of the pedigree, we used SIMWALK 2.0 in a genomic screen with approximately 350 microsatellite markers at less than 20 cM resolution and identified several markers on chromosomes 1, 2, 5, 17, and 18. Using a more stringent requirement of lod score greater than 1.0 in parametric testing and a P value less than 0.5 in a nonparametric test, markers on chromosomes 2 and 18 were identified. The strongest result was with marker D2s2968 (252 cM sex-averaged) in a region near the recently identified region on chromosome 2q36-37 that was recently identified in a genomic screen of sibling pairs with a strong family history. This data suggests that at least one novel locus for PD exists in the Amish population.

**A genome-wide scan for age-related cortical cataracts in a sample from the Beaver Dam Eye Study (BDES) shows evidence of linkage on chromosome 1.** *J.H. Schick<sup>1</sup>, S.K. Iyengar<sup>1</sup>, K. Reading<sup>1</sup>, R. Liptak<sup>1</sup>, C. Millard<sup>1</sup>, K.E. Lee<sup>2</sup>, E.L. Moore<sup>2</sup>, G. Jun<sup>1</sup>, R. Klein<sup>2</sup>, R.C. Elston<sup>1</sup>, B.E. Klein<sup>2</sup>.* 1) Epidemiology & Biostatistics, Case Western Reserve University, Cleveland, OH; 2) Ophthalmology and Visual Sciences, University of Wisconsin Medical School, Madison, WI.

Age-related cortical cataract is a common complex disorder with a multifactorial etiology. The importance of genetics in its etiology has been demonstrated by population, family and twin studies. Although genes for the Mendelian forms of cataracts have been identified, no genome scans or linkage studies have been conducted for age related cataracts. We performed genome-wide linkage scans for cortical cataracts and their rate of change in a selected sample from Beaver Dam, Wisconsin. The Beaver Dam Eye Study (BDES) is a longitudinal study to evaluate risk factors for age related macular degeneration and lens opacities. We genotyped 353 autosomal markers in 325 participants (N = 257 sib pairs). Second stage mapping was conducted in regions that demonstrated marginal evidence of linkage (P 0.01). Multipoint linkage analysis was performed using SIBPAL (S.A.G.E. 4.3) to identify regions linked to this age-related disease. The trait of interest was a quasi-continuous 12-category severity score determined using continuous gradings of duplicate Neitz photographs that included nine segments of the lens of the eye. Prior to conducting the linkage analysis, we adjusted this quasi-quantitative trait for age, age<sup>2</sup>, sex, age x sex interaction and vitamin usage. There was empirical evidence of linkage along a 58 cM region of chromosome 1p at a threshold significance level of 0.01 or less that includes markers D1S1597 (P = 0.0137), GATA29A05 (P = 0.0012), D1S552 (P = 0.0010), and D1S1622 (P = 0.0009). We also found suggestive evidence of genetic linkage for the rate of change over time for this disease along a 12 cM region of 1q21-35 containing markers D1S518 (P = 0.0288), D1S202 (P = 0.0288) and D1S2525 (P = 0.0396). Our analysis suggests that one or more genes for cortical cataract development may exist on chromosome 1, the first step in positional cloning.

**Using genomic convergence to identify susceptibility genes for age-related macular degeneration (AMD).** S.

*Schmidt<sup>1</sup>, E.A. Postel<sup>1</sup>, A. Agarwal<sup>2</sup>, J.R. Gilbert<sup>1</sup>, M.B. Gorin<sup>3</sup>, C. Bowes Rickman<sup>1</sup>, J.L. Haines<sup>2</sup>, M.A. Pericak-Vance<sup>1</sup>.* 1) Duke Univ Med Ctr, Durham, NC; 2) Vanderbilt Univ Med Ctr, Nashville, TN; 3) Univ Pittsburgh School of Med, Pittsburgh, PA.

We have completed a genomic screen of 62 affected sib-pair families with AMD, on whom 389 microsatellite markers were genotyped. Data on 12 follow-up markers are available on an additional 34 multiplex families. We have identified four chromosomal regions of interest: (i) chr 16p11-12 with a non-parametric MLS of 3.5 on in a subset of 14 obese families (average BMI of affected siblings >28.5), which overlaps the linkage region from an independent report; (ii) chr 10q26 with an MLS of 1.6, also consistent with a previous study; (iii) chr 9p24 with an MLS of 1.4 in a subset of 76 non-APOE4 families (APOE4 may be protective for AMD); (iv) chr 1q25-31 with an MLS of 1.3, also consistent with previous reports. To prioritize positional candidate genes within these regions, we are combining our linkage data with independently generated gene expression data. To this end, we have developed a human cDNA microarray database with regional neural retina expression levels for over 9000 genes. The retina expression profiles were generated by differentially screening arrays with two distinct neural retina cDNA probes derived from 4mm trephine punches of pooled (28-66 year old) human neural macula and mid-peripheral retina. 165 genes in the four regions of interest are detected on the array and are hence expressed in the neural retina. 92 of them show elevated expression in the macula. We are analyzing SNP genotype data for identified candidate genes in our current data set of over 650 multiplex and singleton AMD patients, and over 170 unrelated controls with documented absence of AMD. Parallel to our genotyping efforts, we will verify macular expression of these genes by real-time quantitative RT-PCR. Our approach illustrates the emerging paradigm of genomic convergence for dissecting the genetic basis of complex disorders.

**A GENOME-WIDE SCAN FOR LINKAGE IN 42 ANDALUSIAN MULTIPLEX FAMILIES WITH BIPOLAR AFFECTIVE DISORDER.** *J. Schumacher*<sup>1</sup>, *R. Abou Jamra*<sup>1</sup>, *O. Diaz*<sup>2</sup>, *F. Rivas*<sup>2</sup>, *S. Ohlraun*<sup>3, 4</sup>, *R. Kaneva*<sup>5</sup>, *C. Windemuth-Kieselbach*<sup>6</sup>, *E. Gay*<sup>2</sup>, *S. Sans*<sup>2</sup>, *M.J. Gonzalez*<sup>2</sup>, *S. Gil*<sup>2</sup>, *F. Cabaleiro*<sup>2</sup>, *T.F. Wienker*<sup>6</sup>, *P. Nrnberg*<sup>7</sup>, *Y.A. Lee*<sup>7</sup>, *S. Cichon*<sup>8</sup>, *P. Propping*<sup>1</sup>, *M. Rietschel*<sup>3, 4</sup>, *M.M. Nthen*<sup>8</sup>. 1) Inst. of Human Genetics, Univ. of Bonn, Germany; 2) Civil Hosp. Carlos Haya, Mlaga, Spain; 3) Central Inst. of Mental Health, Mannheim, Germany; 4) Dept. of Psychiatry, Univ. of Bonn, Germany; 5) Lab. of Mol. Pathology, Med. Univ., Sofia, Bulgaria; 6) Inst. of Med. Biometry, Informatics and Epidemiology, Univ. of Bonn, Germany; 7) MDC, Berlin, Germany; 8) Dept. of Medical Genetics, Univ. of Antwerp, Belgium.

In order to identify chromosomal regions containing genes that might play a role in determining susceptibility to bipolar affective disorder, we have performed a genome wide screen with 413 STRs in a sample of 42 multiplex families consisting of 357 individuals recruited in Andalusia, Spain. Parametric and non-parametric affecteds-only linkage analyses were carried out under three affection status models: a narrow model which included only individuals with bipolar I (103 affecteds), an intermediate model which included also individuals with bipolar II and schizoaffective disorder (144 affecteds) and a broad model which also comprised individuals with recurrent unipolar depression (209 affecteds). Suggestive evidence for linkage in the non parametric analysis (NPL) were observed in chromosomal regions 1p36, 2p22, 4q31, 6q23-q24, 11q13, and 13p13. Parametric two-point-analysis revealed suggestive LOD-scores on chromosomes 1p36, 4q31, 8p22, 13q13, and 19p13. We are currently performing fine mapping with dense sets of STR markers on chromosomal regions 1, 2, 4, 6 and 13. These regions have previously been described in independent genome screens of BPAD. Our fine mapping sample also includes 66 German families with BPAD because this series of families provided evidence for linkage to these chromosomal regions in a previous genome scan conducted by our group.

**Leber Congenital Amaurosis: evidence for a novel disease-causing gene in a Greek family.** Z. Yang<sup>1, 2</sup>, M.B. Ptersen<sup>3</sup>, N. Faulkner<sup>1, 2</sup>, M. Payne<sup>1, 2</sup>, M. Machan<sup>1, 2</sup>, S. Sims<sup>1, 2</sup>, L. Jiang<sup>1, 2</sup>, X. Li<sup>1, 2</sup>, J. Hu<sup>1, 2</sup>, G. Aperis<sup>3</sup>, M. Grigoriadou<sup>3</sup>, K. Zhang<sup>1, 2</sup>. 1) Department of Ophthalmology and Visual Science, University of Utah Health Science Center, Salt Lake City, UT 80132; 2) Program in Human Molecular Biology and Genetics, University of Utah Health Science Center, Salt Lake City, UT 80132; 3) Department of Genetics, Institute of Child Health, Athens, Greece.

**Purpose:** Leber Congenital Amaurosis (LCA) is a group of autosomal recessive retinal dystrophies representing one of the most severe visual impairments in infants and children. Known Loci/genes for this disorder include 17p13.1 (LCA1, GUCY2D), 1p31 (LCA2, RPE65), 14q24 (LCA3), 17p13.1 (LCA4, AIPL1), 6q11-q16 (LCA5), 14q11 (LCA6, RPGRIP1), 1q31-q32.1 (RP12, CRB1), and 19q13.3 (CRX). We report an eight-generation kindred with recessive Leber Congenital Amaurosis (LCA) without linkage to any of the known loci or associated mutations in previously reported LCA genes. **Methods:** Ophthalmic examination was performed, and genomic DNA was obtained from blood samples after informed consent. Linkage analysis using short-tandem repeat polymorphic markers encompassing known LCA loci and mutational screening of the reported genes for LCA by direct DNA sequence were performed. **Results:** An eight-generation Greek family with a total of 38 members (5 members affected by LCA) was studied. The disease gene was transmitted as an autosomal recessive trait. Of 25 individuals who participated in the study, 5 were found to be affected. Affected individuals showed light perception vision and nystagmus. Fundus examinations revealed diffuse pigmentary changes throughout the retina. Linkage analysis showed that none of the known LCA loci was associated with the phenotype in this kindred. Direct sequencing of GUCY2D, RPE65, AIPL1, CRB1, and CRX revealed no mutations in any of the affected individuals. **Conclusions:** We have identified a large Greek family with LCA. Linkage analysis and candidate gene sequencing have excluded known loci/genes. A genome-wide scan is underway to identify the potentially novel locus associated with LCA in this kindred.

Program Nr: 1904 from 2003 ASHG Annual Meeting

**A nonsense mutation in the I-branching -1,6-N-acetylglucosaminyl-transferase (IGnT) gene, causes autosomal recessive congenital cataract.** *E. Pras, N. Smaoui, JF. Hejtmancik.* OGVFB, National Eye Institute, NIH, Bethesda, MD.

**Purpose:** To identify the genetic defect causing autosomal recessive congenital cataract in 4 Arab Muslim families.

**Methods:** Genotyping was performed using the ABI Prism linkage mapping set (MD10). Two-point lod scores were calculated using MLINK of the LINKAGE Program Package. Mutation analysis of the IGnT gene was performed by direct sequencing of PCR amplified exons.

**Results:** The cataract locus was mapped to a 10.5 cM interval on Chr. 6p24 in between D6S470 and D6S289. Maximum two point lod scores of 7.986 at  $\alpha=0.019$  was obtained for the marker D6S470. Sequencing exons of the IGnT gene, mutation of which have been associated with cataracts and the i blood group phenotype, of these families revealed a homozygous GA substitution in base 58 of exon-2, resulting in the formation of a premature stop codon W328X, W326X and W328X, of the IGnTA,B and C isoforms, respectively. Subsequent blood typing of affected family members confirmed the possession of the rare adult i blood group phenotype.

**Conclusions:** A nonsense mutation in the IGnT gene isoforms causes autosomal recessive congenital cataract in 4 Arab Muslim families. These findings provide further insight into the dual role of the I-branching -1,6-N-acetylglucosaminyl-transferase in the lens and reticulocytes.

Program Nr: 1905 from 2003 ASHG Annual Meeting

**Genetic studies of stuttering in inbred Pakistani families.** *N. Riaz*<sup>1</sup>, *J. Ahmad*<sup>2</sup>, *B. Raza*<sup>2</sup>, *S. Riazuddin*<sup>2</sup>, *N. Cox*<sup>3</sup>, *D. Drayna*<sup>1</sup>. 1) NIDCD/NIH, Rockville, MD; 2) CEMB, University of the Punjab, Lahore, Pakistan; 3) Department of Human Genetics, University of Chicago, Chicago, IL.

Stuttering is a communication disorder that affects speech fluency, and is diagnosed by the presence of syllable repetitions, syllable prolongations, and by interruptions in the smooth flow of speech known as blocks. Many types of evidence suggest genetic factors contribute to stuttering. Because the genetics of stuttering is complex, we have sought specialized populations to give added power to linkage studies. One hundred consanguineous stuttering families were identified from the city of Lahore and surrounding villages in Punjab Province, Pakistan. Blood and diagnostic speech samples were collected from 55 families where there were more than 3 stuttering individuals. These families contain an average of three inbreeding loops containing affected individuals, and 90% of the individuals genotyped are the offspring of either first or second cousin matings. Diagnosis was performed using Stuttering Severity Instrument, and quantitative measures of affection status were obtained. We are undertaking genetic linkage studies using a total of 405 markers from the Weber/Marshfield set 9. A total of 250 individuals have been genotyped, and non-parametric linkage analysis methods are being performed.

Program Nr: 1906 from 2003 ASHG Annual Meeting

**Genome-Wide Scan for Adult Height in a Cohort of Type 2 Diabetes Patients from West Africa.** *J. Zhou, G. chen, Y. chen, H. Daniel, C. Rotimi.* Natl Human Genome Ctr, Howard Univ, Washington, DC.

A linkage analysis was performed for adult height in a cohort of diabetes patients from five centers in West Africa (Ghana: Accra and Kumasi; Nigeria: Enugu, Ibadan and Lagos). Using the variance component approach in SOLAR package, we searched for informative genomic regions for adult height (cm) in 341 families including 691 diabetes cases. Kurtosis for adult height in this cohort was 1.7. The strongest evidence of multipoint linkage analysis was obtained at 112cM (marker D3S4529) on chromosome 3 (LOD = 2.74, P-value = 0.00019). Follow by a LOD = 2.23 (P-value = 0.00068) at 53cM (marker D6S2427) and a LOD score = 1.84 (P-value = 0.0018) at 155cM (marker D6S2436) on chromosome 6, a LOD score = 1.82 (P-value = 0.0019) at 140cM (marker D8S1128) on chromosome 8, and a LOD score = 2.11 (P-value = 0.00092) at 88cM (marker D16S2624) on chromosome 16. All analyses were adjusted for gender ( $P < 0.0000001$ ) and age ( $p = 0.0000041$ ). The linkage signals lie in regions that have been reported in several previous studies conducted in multiple populations and ethnic groups. In conclusion, we replicated linkage evidence for adult height in chromosomes 3, 6, 8, and 16 in a cohort of diabetes patients from West Africa one of the ancestral populations of African Americans.

**A genome-wide scan for endometriosis loci in a Finnish study sample.** *O. Saranen*<sup>1,2</sup>, *M. Perola*<sup>2,3</sup>, *L. Heinonen*<sup>2</sup>, *K. Pelli*<sup>2</sup>, *J. Papp*<sup>2</sup>, *M. Ryyanen*<sup>1</sup>, *L. Peltonen*<sup>2,3</sup>. 1) Department of Obstetrics and Gynecology, University of Oulu, Oulu, Finland; 2) Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, USA; 3) Department of Molecular Medicine, National Public Health Institute, and Department of Medical Genetics, University of Helsinki, Helsinki, Finland.

Endometriosis is a common gynecological disorder among fertile women with a prevalence of 10-15 %. Several twin studies have shown evidence for a genetic trait in this disease, with genetic risk ratios similar (2-3) to other complex disorders such as asthma or diabetes. However, no significant linkage evidence for any chromosomal region has been reported so far. Here we describe a genome-wide linkage study of endometriosis in nuclear families originating from the genetically isolated Finnish population. We have performed a two-stage genome-wide scan in a Finnish endometriosis study sample consisting of 31 core families, each with multiple affected subjects (178 individuals, 83 are affected women). All diagnoses were confirmed via laparoscopy or laparotomy and unaffected individuals were used only for phase information. In stage I we genotyped 323 markers (Weber 9 screening set) and in stage II the regions of interest were covered with denser marker maps. Further, 9 additional core families were included in the study in stage II. We identified suggestive linkage evidence for loci on chromosomes 7q ( $Z=1.98$ ), 8q ( $Z=2.35$ ) and 1p ( $Z=2.57$ ). These regions are currently being studied in the set of families ascertained for their geographical region of origin or shared genealogical roots to maximize the potential for shared genetic background in the subisolates of Finland.

**Genetic heterogeneity in familial chordoma.** *R.X. Yang<sup>1</sup>, M.J. Kelley<sup>2</sup>, M. McMaster<sup>1</sup>, N.J. Liebsch<sup>3</sup>, A.W. Bergen<sup>4</sup>, M. Beerman<sup>4</sup>, K. Haque<sup>4</sup>, A.M. Goldstein<sup>1</sup>, D.M. Parry<sup>1</sup>.* 1) Dept DCEG, NCI, NIH, DHHS, Rockville, MD; 2) Dept. of Medicine, Duke Univ., Durham, NC; 3) Dept. of Radiation Oncology, Mass. General Hospital, Boston, MA; 4) CGF, NCI, NIH, DHHS, Gaithersburg, MD.

Chordoma is a rare tumor originating from notochordal remnants. Five chordoma families with two or more affected individuals have been described, suggesting a genetic predisposition. The pattern of inheritance in these chordoma families is compatible with an autosomal dominant trait. Previously, we performed a whole genome scan on three unrelated chordoma kindreds (16 patients with chordoma) and reported significant evidence for linkage to chromosome 7q33 ( $Z_{\max} = 4.78$ ) with a minimal disease locus region of 11 cM. In the present study, a new chordoma family from Italy was clinically evaluated and genotyped for microsatellite markers on chromosome 7q. The father had nasopharyngeal chordoma at age 9 that was treated with radiation therapy. The tumor recurred at age 42 and he died at age 47 from tumor progression. One daughter was diagnosed with skull base chordoma at age 7, the second daughter had multicentric chordoma diagnosed at age 25, and the third daughter was diagnosed with a cervical astrocytoma at age 12. The three daughters and their mother were genotyped for 16 chromosome 7q markers from the chordoma locus region previously identified. The two daughters with chordoma did not share the same paternal haplotype for these 7q markers, suggesting the possibility of genetic heterogeneity. We cannot exclude the possibility that one of the affected daughters had a sporadic chordoma. However, since chordoma is such a rare tumor, this is highly unlikely. To further investigate the evidence for linkage and genetic heterogeneity in this region, we genotyped these four chordoma families for 120 SNP markers on chromosome 7. Examination of these SNP markers in conjunction with the microsatellite markers should help resolve the question of genetic heterogeneity of familial chordoma.

Program Nr: 1909 from 2003 ASHG Annual Meeting

**A Genomic Screen of Essential Tremor.** *F. Zhang, J. Stajich, S. West, R. Smith, J. Vance, M. Pericak-Vance, A. Ashley-Koch, J. John.* Duke Univ Medical Ctr, Durham, NC.

Essential tremor (ET), the most common movement disorder in adults, is clinically and genetically heterogeneous. ET is characterized by postural and intention tremor primarily affecting the arms and hands, although the head, voice, and legs may also be affected. Segregation analysis has suggested that the inheritance of ET is autosomal dominant and that the penetrance is nearly complete by the age of 65 (Bain et al, 1994). To date, three ET loci have been reported on chromosomes 2, 3 and 4. Evidence from our own dataset and that of Kovach et al (2001) has suggested that additional, unidentified ET loci exist. To that end, we have performed a 12 cM microsatellite genomic screen of a large ET family comprised of 36 sampled individuals, 9 of whom are affected. Two-point linkage analysis was performed with the VITESSE software package. Based on these results, high-priority regions on chromosomes 5, 8, 15, 16 and 20 have been defined, the two-maximum two-point LOD scores are respectively 1.76, 1.89, 1.63, 1.35, and 1.25. With additional markers genotyped in a 15 cM region on Chromosome 5, we conducted multipoint analysis. The highest LOD in Chromosome 5 is 2.64. We are currently genotyping additional markers in the rest of high-priority regions for pursuing fine-mapping and multipoint analysis. To assist in localization and fine-mapping of both novel and existing ET loci, we have amassed a collection of over 25 multiplex families. These families will be critical for identifying and isolating ET genes, which will advance therapeutic interventions for this major public health concern.

**Linkage confirmation of renal disease in systemic lupus erythematosus.** *A.I. Quintero-Del-Rio<sup>1,2</sup>, J.A. Kelly<sup>1</sup>, C.P. Garriott<sup>1</sup>, J.B. Harley<sup>1,2,3</sup>*. 1) Oklahoma Medical Research Foundation; 2) University of Oklahoma; 3) VA Hospital, Oklahoma City, OK, United States.

Renal disease occurs in 40-75% of systemic lupus erythematosus (SLE) patients, contributing to much of the morbidity and mortality of SLE. We have previously reported the results of two pedigree stratification strategies that explored the impact of the ACR renal criterion (cellular casts or proteinuria) for SLE classification upon genetic linkage. A multipoint sib-pair regression algorithm (SIBPAL2) produced  $p = 0.0000008$  in European-American (EA) pedigrees at 10q22.3 (SLEN1) and  $p = 0.000001$  in African-American (AA) pedigrees at 2q34-35 (SLEN2). To confirm these previously identified linkage effects, we collected an additional 144 pedigrees multiplex for SLE, 89 of which contained at least one SLE affected with the SLE renal criterion (27 AA, 43 EA, and 19 Hispanic (HI) pedigrees). SIBPAL results confirmed the previously identified SLE susceptibility locus (SLEN2) in the AA pedigrees ( $p=0.002$ ). When both independent sets of pedigrees were analyzed together, a  $p=0.0000006$  was obtained. The SLEN1 effect at 10q22.3 was highly supported by the additional set of pedigrees with a  $p=0.001$  in the same region. In addition, an effect at 5p16.3 is now significant in the AA pedigrees and is seen in both independent groups of pedigrees ( $p=0.00002$  in the initial pedigrees,  $p=0.001$  in the confirmatory pedigrees and  $p=0.0005$  in the combined set). All of the effects found dominated in either the AA or EA and were less impressive in the combined group. Multiple genetic linkages are related to renal criterion in SLE. Confirmations and additionally support of these identified genetic linkages will hopefully lead to the identification of the genes that predispose a lupus patient to develop renal disease.

**Replication of linkage on 2p for chronic mucocutaneous candidiasis and thyroid disease using two different high-density SNP genome scan technologies.** *Y. Tsai<sup>1</sup>, E.W. Pugh<sup>1</sup>, P. Boyce<sup>1</sup>, K.F. Doherty<sup>1</sup>, Y.T. Fan<sup>1</sup>, A.F. Scott<sup>2</sup>, M.St. Hansen<sup>3</sup>, A. Oliphant<sup>3</sup>, H. Loi<sup>4</sup>, R. Mei<sup>4</sup>, J.M. Puck<sup>5</sup>.* 1) CIDR, IGM, JHUSOM, Baltimore, MD; 2) IGM, JHUSOM, Baltimore, MD; 3) Illumina, San Diego, CA; 4) Affymetrix, Inc. Santa Clara, CA; 5) GMBB, NHGRI, NIH, Bethesda, MD.

Previously Atkinson et al. (AJHG 69:791-803) identified a candidate linkage region on chromosome 2p using a 10 cM microsatellite genome scan in a family with a combination of chronic mucocutaneous candidiasis and thyroid disease. We attempted to replicate this linkage result using single nucleotide polymorphism (SNP) markers and two different detection technologies, Illumina's BeadArray™ platform Linkage Panel Version 1 and Affymetrix GeneChip Human Mapping 10K Array Xba 130 (Early Access version). Data from 18 samples from this family was received from Affymetrix and Illumina. The overall missing data rate for this family was 0.022% using Illumina 2,245 SNPs, and 3.72% for Affymetrix 8,803 SNPs. The Mendelian inconsistency rate was 0.03% for both technologies. Genehunter was used for nonparametric linkage analysis. Due to this program's constraints, a trimmed version of the pedigree containing 20 individuals and 11 genotyped individuals was used. Genetic locations were approximated based on the physical map. For multipoint analysis we excluded non-informative markers (two point NPL scores between -0.05 and 0.05). The highest two point NPL score on chromosome 2 using Illumina assay (193 SNPs) was 0.56 (p=0.076) at position 45.29 cM and using Affymetrix assay (717 SNPs), was 0.81 (p=0.05) at positions 70.84 and 74.33 cM. Among the 190 informative Illumina SNPs, multipoint NPL scores reached 2.17 (p=0.035) in the 56.92-80.33 cM region on chromosome 2. For the 392 informative Affymetrix SNPs, multipoint NPL scores reached 9.41 (p=0.0078) in the 44.21-58.16 cM region. Original results using microsatellite markers reported parametric two point LOD scores greater than 1.0 at 5 consecutive markers in the 38.33-73.61 cM region. In this family, our results from both SNP technologies were comparable to that of the original microsatellite genome scan. Additional results using Illumina Linkage Panel Version 3 will be provided.

**Evidence for linkage to chromosome 19 in autism.** *M.A. Pericak-Vance<sup>1</sup>, R. Rabionet<sup>1</sup>, D. Skaar<sup>1</sup>, R.K. Abramson<sup>2</sup>, H.H. Wright<sup>2</sup>, G.R. DeLong<sup>1</sup>, M.L. Cuccaro<sup>1</sup>, J.R. Gilbert<sup>1</sup>.* 1) Duke University Medical Center, Durham, NC; 2) University of South Carolina, Columbia, SC.

Previous genome-wide screens for autism have provided suggestive evidence for linkage to Chromosome 19, including our own, in which 99 multiplex families were analyzed (multipoint maximum LOD score (MLS)=1.21). In an effort to confirm and expand these findings we conducted a follow-up analysis of the region of observed linkage in 210 multiplex families. The families were ascertained through the Collaborative Autism Team (CAT) (N=111) and through the Autism Genetics Exchange (AGRE) (N=99) and met ADI and ADOS criteria for the diagnosis of autism. Five microsatellite markers spanning a region of chromosome 19 from 45.5cM to 58.6cM (d19S593, d19S407, d19S407, d19S49, d19S433) were analyzed. The most significant result was for marker d19s593 with a maximized parametric lod score allowing for heterogeneity (MLOD) of 2.5 (recessive model), and an MLS of 2.0 in the overall dataset. Stratifying the families by ascertainment source (CAT vs. AGRE), however, resulted in a significant result in the AGRE subset (MLOD=4.1 and an MLS=3.2) for D19S593. D19S407 located 3cM distal was also positive with MLOD=1.0. These results continue to strongly implicate this region of chromosome 19 as containing an autism susceptibility gene and suggest that family source is an important stratification variable to consider in analysis. High resolution SNP mapping of the region is ongoing and will be presented.

**Identifying modifier loci in existing data.** *E.W. Daw, S. Shete.* Dept Epidemiology, UT MD Anderson Cancer Center, Houston, TX.

In many disorders where a primary disease-causing locus has been identified, evidence for additional trait variation due to genetic factors has been found. These findings have led to several studies in which secondary modifier loci are being sought. Identification of such modifier loci provides additional insight into disease mechanisms and may provide additional treatment targets. We believe that some modifier loci can be identified by re-analysis of genome screen data while controlling for the effects of the primary locus. To test this hypothesis, we simulated multiple replicates of typical genome screening data on to two real family structures that were used to identify a genetic mutation that causes hypertrophic cardiomyopathy. Along with the marker data, we simulated a trait with characteristics similar to one measure of hypertrophic cardiomyopathy. This trait was influenced by a primary gene, a secondary modifier gene, and a third very small effect gene. We examined the power and false positive rates to map the secondary locus while controlling for the effect of the primary locus with three types of analyses. First, we examined the L-scores produced by the Monte Carlo Markov chain (MCMC) combined segregation and linkage analysis package, Loki. These L-scores are Bayes Factors for linkage computed in 1cM intervals, with a score 1 suggesting linkage and 1 suggesting no linkage. Second, we examined an alternate scoring method for MCMC linkage analysis, the LOP (Daw et. Al., Genetic Epidemiology 24:181-190), which is the log of the posterior probability of linkage to the real chromosome divided by that to an unlinked pseudochromosome. Third, we calculated lod scores using an individual-specific liability class based on the quantitative trait value. We found that all three methods produced scores that are significant on a genome-wide level in some replicates. Our results indicate that a re-analysis that accounts for the effect of an identified gene may localize modifier genes. In view of the relatively low cost of such analysis, we recommend re-analysis of exiting genome screen data as a first step in hunting for modifier genes. Support: HL68884.

**How useful is fine mapping complex trait linkage scan peaks ?** *S. Wiltshire, A. Morris, M. McCarthy, L.R. Cardon.*  
Wellcome Trust Ctr Human Gen, Univ Oxford, Oxford, United Kingdom.

Genotyping additional markers under linkage peaks in primary genome scans is a near universal practice. Strongly implicit inferences regarding the true or false nature of a linkage result are often made on the basis of the behaviour of the peak upon fine mapping: if the peak increases, it is thought to be a true positive; if it decreases, it is regarded as a possible false positive. We have quantified the statistical inferences that can be made from such exercises by simulation. With completely genotyped data and no gene effects (ie., the null hypothesis of no linkage), increased support for a false positive linkage from a primary analysis occurs 54.2% of the time upon fine mapping with a LOD threshold 1.18. With a threshold of LOD2.08, such a peak increases 48.6% of the time. With a real gene in the linkage region (eg., with  $\lambda_S=1.25$ ), increased evidence for a true positive linkage result occurs 74.7% of the time with a fine mapping LOD threshold 1.18, and 70.1% of the time with LOD threshold 2.08. Given the simple instance of a 5% prior probability for linkage to a causative gene, the posterior probabilities of a true positive before fine mapping are 0.18 (LOD1.18) and 0.49 (LOD2.08). If the peak increases on fine mapping, these posterior probabilities increase to 0.24 (LOD1.18) and 0.58 (LOD2.08; if it decreases on fine mapping, these drop to 0.11 (LOD1.18) and 0.36 (for LOD2.08). As  $\lambda_S$  increases, these posteriors increase as the linkage peak increases and decrease as the peak shrinks, on fine mapping. When both the priors and the LOD threshold for fine mapping increase, both posteriors increase. Similar results are obtained under different data configurations (i.e. absence of parents, and/or otherwise incomplete genotypes). In summary, there are only modest increases in the probability that a linkage peak reflects a real gene when LOD scores increase, and modest decreases when the peak shrinks, upon fine mapping. Peak behaviour upon finemapping would therefore appear to add little to determining whether a linkage peak is a true or a false positive, the principal determinant of which remains the magnitude of the primary LOD score.

Program Nr: 1915 from 2003 ASHG Annual Meeting

**Representing Quantitative Trait Loci in the Mouse Genome Informatics (MGI) Database.** *D.W. Bradt, I. Lu, J.T. Eppig, C.J. Bult, Mouse Genome Informatics Staff.* Bioinformatics, The Jackson Laboratory, Bar Harbor, ME.

The Mouse Genome Informatics (MGI) database is a community database that provides integrated access to data on the genetics, genomics, and biology of the laboratory mouse. MGI is freely available on the Web at <http://www.informatics.jax.org/>. Mapping information in MGI is obtained from primary research data collected reported in scientific publications as well as electronically submitted data. Genetic linkage data specify parental genotypes and progeny segregants. Mapping data includes representation of Quantitative Trait Loci (QTL) derived from published literature. Information about each QTL is available as free text and includes the genetic marker with the peak statistical association score. If available in the associated publication, the information about flanking markers and candidate genes are described. Progenitor strain polymorphisms underlying the mapping of the QTLs are also detailed. Genetic map positions for QTLs expressed as cM positions based on the marker with which the QTL has the highest reported linkage. Information on approximately 1,400 QTLs are included within MGI currently. QTL from MGI have been integrated with the public mouse genome sequence. The QTLs displayed in their genomic context can be observed via the Ensemble genome browser, the UCSC Genome Browser, and NCBI's Map Viewer. The integration of QTL data from MGI with the annotated mouse genome serves as a useful framework for in silico positional candidate gene analysis.

**Genome-screen of a healthy cohort of twin pairs reveals linkage for bone metabolism markers to chromosomes 1, 12, 17 and 19.** *T. Andrew<sup>1</sup>, M.J. Barber<sup>1</sup>, R. Swaminathan<sup>2</sup>, M. Langdown<sup>3</sup>, S.G. Wilson<sup>4</sup>, T.D. Spector<sup>1</sup>.* 1) *Twin Research & Genetic Epidemiology Unit, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH;* 2) *Department of Chemical pathology, St.Thomas' Hospital;* 3) *Sequenom Inc., 3595 John Hopkins Ct, San Diego, CA 92121;* 4) *Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Western Australia 6009.*

**Purpose:** Osteoporosis is a disease characterized by low bone mass, architectural deterioration of bone tissue and abnormalities in bone turnover. In this study we have conducted a genome-wide scan for bone turnover markers previously reported to have high heritabilities.

**Methods:** Five genome-wide screens were conducted using upto 932 dizygous caucasian female twin pairs aged between 18 and 80 years. Levels of blood serum total and bone specific alkaline phosphatase were used as distal measures of bone formation; Urinary deoxypyridinoline crosslinks (DPD) for bone formation; and serum parathyroid hormone and serum 25-hydroxyvitamin D (25(OH)D) for bone calcium and vitamin D regulation, respectively. Multipoint linkage analyses were performed using optimal Haseman and Elston methods implemented in Stata as a Generalised Linear Model. Regression diagnostics were used to help identify poor fitting linkage models and hence artefactual results.

**Results:** Linkage peaks were observed for 25(OH)D at chr1p21-31 (LOD 3.1-4.2), chr17q21-24 (LOD 2.2-4.5) and chr19q13.4 (LOD 5.4-7.1); DPD peaked at chr12q24.3 (LOD 4.3-4.7).

**Conclusion:** Preliminary evidence for bone markers linked to chromosomes 1, 12, 17 and 19 highlight these regions as potentially important for studies of genes that regulate bone.

**Findings in an independent sample support association between bipolar affective disorder and the G72/G30 locus on chromosome 13q33.** *Y.-S. Chen<sup>1</sup>, N. Akula<sup>2</sup>, S.D. Detera-Wadleigh<sup>2</sup>, T.G. Schulze<sup>3</sup>, J. Thomas<sup>2</sup>, J.R. DePaulo<sup>4</sup>, M.G. McInnis<sup>4</sup>, N.J. Cox<sup>1</sup>, F.J. McMahon<sup>2</sup>.* 1) Human Genetics, University of Chicago, Chicago, IL; 2) Mood and Anxiety Program, National Institute of Mental Health Intramural Research Program, National Institutes of Health, Dept. of Health and Human Services, Bethesda, MD; 3) Division of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany; 4) Dept. of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, MD.

Markers near the genes G72 and G30 on chromosome 13q33 have been implicated in the etiology of schizophrenia and, recently, bipolar affective disorder (BPAD). Hattori et al (2003) reported that single-nucleotide polymorphisms (SNPs) near the G72/G30 locus were associated with BPAD in a small pedigree sample and that SNP haplotypes were associated in a second, larger sample of triads. The present study attempts to replicate this finding in an independent case-control sample. Eight SNPs near the G72/G30 locus, including the most strongly associated markers in the previous study, were genotyped in 139 cases and 113 ethnically-matched controls. Significant association was detected between BPAD and 2 adjacent SNPs (smallest  $p = 0.007$ ; global  $p=0.024$ ). Haplotype analysis produced additional support for association (smallest  $p = 0.004$ ; global  $p=0.004$ ), although the associated haplotypes differ from those previously reported. These results support the previous findings and provide further evidence, in an independent sample, for an association between BPAD and genetic variation in the vicinity of the genes G72 and G30.

**Quantitative Trait Loci for steady-state platelet count in mice.** *M. Buckley*<sup>1,2</sup>, *C. Cheung*<sup>1,2</sup>, *I. Martin*<sup>3</sup>, *K. Zenger*<sup>4</sup>, *J. Donald*<sup>4</sup>, *P. Thompson*<sup>3</sup>, *C. Moran*<sup>3</sup>. 1) Centre for Vascular Research, University of New South Wales, Sydney, Australia; 2) Molecular & Cytogenetics Unit, South Eastern Area Laboratory Services, Sydney, Australia; 3) Centre for Advanced Technologies in Animal Genetics and Reproduction, University of Sydney, Australia; 4) Department of Biological Sciences, Macquarie University, Sydney, Australia.

Approximately 85 percent of the inter-individual variance in human platelet numbers can be attributed to genetic factors. As part of a project to identify novel factors that regulate platelet count, we identified two inbred mouse strains, CBA/CaH and IQS5, with substantial differences in platelet count (mean values of 581 vs. 1062 x 10<sup>9</sup>/L). An F2 intercross resource of 1,126 animals was bred for a genome-wide scan for quantitative trait loci (QTL) for platelet count. QTL were identified on MMU 1 (LOD 6.8, p<0.0005) and MMU 11 (LOD 11.2, p<0.0005) by selectively genotyping animals from the extremes of the F2 platelet count distribution. Three other QTL of borderline significance were also detected. It is noteworthy that no QTL was detected in the vicinity of the genes encoding thrombopoietin (Tpo), and its receptor (c-Mpl), both known to influence platelet production. These results represent the first published use of a genetic linkage based approach to the identification of genetic factors that regulate platelet count.

MMU chr	11	1	7	13	17
Position (cM)	45	94	24	53	20
% Variance	9.2	5.6	4.1	4.3	3.3
Change in PLT	+48 (x10exp9)	-36	+30	+29	+28
LOD Score	11.2	6.8	3.6	3.4	3.5

**Refinement of the 6p21.3 QTL influencing dyslexia through linkage and association analysis.** *K.E. Deffenbacher<sup>1</sup>, J.B. Kenyon<sup>1</sup>, D.M. Hoover<sup>1</sup>, R.K. Olson<sup>3</sup>, B.F. Pennington<sup>2</sup>, J.C. DeFries<sup>3</sup>, S.D. Smith<sup>1</sup>*. 1) Dept Molec Genetics, Univ Nebraska Med Ctr, Omaha, NE; 2) Univ of Denver, Denver, CO; 3) Institute for Behavioral Genetics, Univ of Colorado, Boulder, CO.

Reading disability (RD), also known as dyslexia, is the most common learning disability with a prevalence rate of ~5-10% in school age children. RD is known to be highly heritable with evidence of a neurobiological origin. A quantitative trait locus (QTL) for RD has been reported on chromosome 6p21.3, spanning a 16.4Mb (13.8cM) interval from D6S109-D6S291 (Fisher and DeFries 2002). In this study, we performed sib pair linkage analyses using Haseman-Elston and DeFries-Fulker methods to define more accurately the QTL interval. An extended sample of 349 nuclear families were genotyped for 22 microsatellite markers spanning ~22Mb of the 6p21-22 region. Linkage was assessed using five quantitative phenotypes, including a composite measure of reading performance and 4 component phenotypes. When probands were selected for severe scores, single and multipoint analysis showed suggestive linkage with 3 of our phenotypes, converging over an ~3.4Mb interval spanning D6S1597-D6S1571. Multipoint analysis suggested a peak of linkage for the composite measure of reading ability near D6S1663, with 2 of the component phenotypes showing a peak near D6S1571. Out of the twelve genes in the linkage interval, ten cluster within ~680kb, and were selected for association analysis based on CNS expression and putative function. 31 SNPs spanning these 10 genes were genotyped in 114 family trios. Marker-trait associations were assessed using QTDT and FBAT, and haplotype analysis was done using Genehunter TDT in conjunction with 1 of the 3 linked phenotypes. Marker associations were detected in four of the ten genes, results that were corroborated by our haplotype TDT analysis. The results of the association study have thereby allowed us to significantly reduce the number of possible candidate genes, as well as prioritize genes for further mutation screening. This work was supported in part by NICHD Center Grant HD-27802.

**A genome-wide scan for loci contributing to population variation in adult height.** J.A. Ellis, Z.Y.H. Wong, S.B. Harrap. Dept Physiology, Univ Melbourne, Melbourne, Australia.

Adult height is widely regarded as the complex human trait most amenable to genetic dissection. It shows a high degree of heritability (between 55%<sup>1</sup> and 90%), and can be easily and accurately measured. Investigation of this trait therefore has the potential to shape strategies for genomic discovery. Identification of genes controlling height will also enhance our understanding of the fundamental biological processes of bone growth. Several genome-wide linkage analyses of height have recently been published, however few loci have been consistently identified across studies. We have performed a multipoint quantitative genome-wide linkage analysis for height in the Victorian Family Heart Study (VFHS)<sup>1</sup>, a population survey of 2959 healthy Caucasian individuals from 783 adult families. A total of 275 sibling pairs aged 18-31 yrs were genotyped using the ABI Linkage Mapping Set v2 that defines a human index map of ~10 cM intervals. Data was analysed using GENEHUNTER 2. Six promising linkage peaks were observed, including Chromosomes 3 (50-80 cM, Z=3.2), 2 (155-185cM, Z=2.7) and X (170cM-tel, Z=2.2). The identified regions have not been reported previously in other genome-wide analyses. However, a number of candidate genes exist in our regions. On chromosome 3, these include the genes encoding parathyroid hormone receptor 1 (*PTHRI*) that was recently associated with height in women<sup>2</sup>, and tetranectin (*TNA*), an extracellular matrix protein produced by osteoblasts. The X-chromosome region contains biglycan (*BGN*), a proteoglycan synthesised by osteoblasts. The next step is to undertake a thorough association analysis of single nucleotide polymorphisms (SNPs) in and around candidate genes, and if necessary, throughout identified regions to elucidate the causative variants. It will not be until such comprehensive investigations of the results of genome-wide scans of complex traits are carried out that we will begin to fully understand the accuracy and reliability of such methods to detect contributing loci, and the basis for the between-study discrepancies. <sup>1</sup> Harrap SB, et al. Am J Epidemiol 152:704-15,2000. <sup>2</sup> Minagawa M, et al. J Clin Endocrinol Metab 87:1791-6,2002.

**A genomewide scan for the susceptibility gene loci to ankylosing spondylitis in Chinese Han population.** *M. Gu*<sup>1</sup>, *W. Yuan*<sup>2</sup>, *J. Yang*<sup>3</sup>, *J. Zhang*<sup>2</sup>, *F. Yao*<sup>3</sup>, *Z. Wang*<sup>1</sup>, *L. Jin*<sup>2,4</sup>, *W. Huang*<sup>2</sup>, *L. Fan*<sup>3</sup>. 1) Department of Medical Genetics, Shanghai 2nd Med. University, Shanghai, China; 2) Chinese National Human Genome Center at Shanghai, Shanghai, China; 3) Shanghai Second Medical University and Shanghai Institute of Immunology, Shanghai China; 4) Center for Genome Information, Department of Environmental Health, University of Cincinnati.

Ankylosing spondylitis (AS) is a common arthritis, with a prevalence of 1/1000-3/1000 in Caucasian and 2/1000 in Chinese population. Genomewide scans showed that some affected-sibling-pair families with AS not only demonstrated strong linkage to the MHC locus, but also identified other regions with suggestive or strong linkage on chromosomes 1p, 2q, 9q, 10q, 16q, and 19q. In order to localize the regions containing genes that determine susceptibility to AS in Chinese, we performed a genome-wide scan in nine Chinese families with AS, including 29 affected individuals. LINKAGE and GNEHUNTER packages were used for parametric (LOD) and non-parametric (NPL) analyses. The significant two-point LOD score value with D6S276 (at 44.9cM from the 6p telomere) was 3.8782 in parametric analysis. Fine mapping showed LOD scores of D6S1691 (at 42.7cM) and D6S1618 (at 47.6cM) around D6S276 were 1.5717 and 2.0056, respectively. Single point NPL score of D6S276, D6S1691 and D6S1618 were 2.6053, 2.7490, 2.0202, respectively, and minimum P value were 0.0072, 0.0047, and 0.0265, respectively. Using multipoint NPL, the maximum LOD score values, NPL score and minimum P value obtained near D6S276 were 5.0623, 3.7561, and 0.000233, respectively. The haplotype analysis showed that the regions from D6S1691 to D6S1618 associated with AS. As a result, the strong linkage of the D6S276 with AS was found, the region of D6S1691-D6S276-D6S1618 exist a susceptibility gene of AS. In addition, we identified 3 regions with suggestive linkage on D3S1292, D4S1535 and D18S64.

**A Leu184Val polymorphism in PCK1 gene is associated with type 2 diabetes in Chinese population.** *Y. Dong*<sup>1,2</sup>, *G. Li*<sup>2</sup>, *G. Wu*<sup>2</sup>, *L. Jin*<sup>1</sup>, *Y. Shen*<sup>1</sup>, *T. Luo*<sup>2</sup>, *M. Luo*<sup>2</sup>, *W. Huang*<sup>1</sup>. 1) Chinese National Human Genome Center at Shanghai, Shanghai, China; 2) Shanghai Institute of Endocrinology, Ruijin Hospital, Shanghai Second Medical University.

Type 2 diabetes mellitus is a multifactorial, heterogeneous metabolic disease with environmental and genetic components. In the previous study, we performed a genome-wide linkage study in Chinese pedigrees with type 2 diabetes and gave a suggestive evidence for linkage to type 2 diabetes, which is on chromosome 20q13. PCK1 gene, as one potential candidate gene within this linked area, is a regulated enzyme catalyzing the conversion of oxalacetate to phosphoenolpyruvate in gluconeogenesis. In this study, we detected the single nucleotide polymorphisms (SNPs) in the coding and regulating regions of the PCK1 gene by direct sequencing and eight SNPs were detected in PCK1 gene, among which one SNP (Leu184Val) was a missense variant. The frequency of the Leu184Val polymorphism was further evaluated in a case-control study (195 type 2 diabetes patients and 188 controls). The allelic frequency of the Leu184Val polymorphism in the cases and controls was 75.6% and 83.2% respectively ( $P=0.009$ ). The odds ratio for the frequency of the mutation allele is 1.6 (the 95%- confidence interval: 1.121~ 2.284). Our data implicated that the Leu184Val polymorphism in PCK1 gene was significantly associated with the pathogenesis of type 2 diabetes in the Chinese population.

Program Nr: 1923 from 2003 ASHG Annual Meeting

**Evidence for linkage but not association to the GABRB3 region of chromosome 15 in a subset of primary open angle glaucoma (POAG) families.** *F.L. Graham<sup>1</sup>, R.R. Allingham<sup>1</sup>, M. Hauser<sup>1</sup>, K. LaRocque-Abramson<sup>1</sup>, C.M. Santiago<sup>1</sup>, A. Ventura<sup>1</sup>, E. DelBono<sup>2</sup>, J.R. Shi<sup>3</sup>, J.L. Haines<sup>3</sup>, J.L. Wiggs<sup>2</sup>, M.A. Pericak-Vance<sup>1</sup>.* 1) Medicine, Duke University Medical Center, Durham, NC; 2) Massachusetts Eye and ear Infirmary, Boston, MA; 3) Vanderbilt University, Nashville, TN.

Previously, we employed Ordered Subset Analysis (OSA) to identify a homogenous subset of POAG families linked to chromosome 15q in order to refine our candidate region for analysis (Allingham, et al, ASHG 2002). We used *age at diagnosis* as a surrogate for age of onset as a covariate in the analysis. The peak OSA lod score reached a maximal value of 3.05 in a subset of 15 families with a family specific mean age at diagnosis 49 years of age at the GABRB3 locus (12.4 cM) ( $p = 0.011$  by permutation test). Within this region are located three GABA receptor genes, GABRA5, GABRB3 and GABRG3. These genes are all expressed in the retina making them good candidate susceptibility genes. Using the 15 families (92 individuals) that were identified by OSA as being a homogeneous subset of the POAG data set, we analyzed 21 single nucleotide polymorphisms (SNPs) within the GABRB3 gene and in the region located beyond the 3' end of the gene. Seven of the twenty-one SNPs showed positive linkage with a peak LOD score of 1.63 for SNP SNPEX1A-2. Allelic association analysis was not significant for any of the SNPs using the Pedigree Disequilibrium Test (PDT) ( $p < 0.05$ ). The full coding region of all three genes were sequenced and no coding variants were found. We are testing other candidate genes as well as additional SNPs in the GABRB3 gene region. Support: The Glaucoma Research Foundation, Barkhouser Glaucoma Research Fund, and NIH Grant EY 10886.

**Genetic variation in a haplotype block spanning IDE has a major impact upon clinical features of Alzheimer's Disease.** *A.J. Brookes, L. Feuk, J. Prince.* Center for Genomics and Bioinformatics, Karolinska Institute, Stockholm, Sweden.

Linkage studies have identified a large (> 60 Mb) region on chromosome 10q that segregates with Alzheimers Disease (AD), but the causative gene in this region remains elusive. In fact, different linkage peak localizations suggest that more than one gene in the region may influence the disease. One candidate gene for which a good biological case could be made is that for insulin degrading enzyme (IDE), which acts to degrade both the extracellular beta-amyloid protein constituent of amyloid plaques and the intracellular domain of APP that is released by gamma-secretase processing. To evaluate IDE in AD, we undertook extensive SNP association analysis in its vicinity (480kb surrounding the gene). Estimations of linkage disequilibrium amongst 26 tested SNPs revealed a major 276kb haplotype block that extends across three genes (IDE, KNSL1, and HHEX). We evaluated this block further by scoring 3 haplotype-tagging SNPs. Experiments employed multiple independent sets of clinical materials comprising early- and late-onset AD individuals (collaborations with Kaj Blennow, University of Gteborg; Boo Johansson, University of Gteborg ; Margaret Gatz, University of Southern California; Nancy Pedersen, Karolinska Institute), and we focused our analyses upon case-control status as well as various quantitative traits that are pertinent to AD diagnosis and severity (MMSE scores, Tau protein levels in CSF, and age-at-onset). Significant evidence of AD association was found, entailing consistent risk and protective haplotypes present in most of the investigated populations (p-values ranging from 0.05 to  $<1 \times 10^{-9}$ ). Thus, strong association was found between features of AD and a haplotype block that spans IDE. The signal patterns seen in our studies, and in collaborative replications of our work (Steven Younkin, Mayo Clinic; Rudi Tanzi, Harvard University) indicate that many allelic variants pathogenic for AD probably exist in and around this genomic domain. Deciphering the etiology of AD encoded by this portion of chromosome 10q will probably require extensive and detailed further study of this challenging genomic region.

**Strong linkage evidence for a locus influencing sensitivity to *Chlamydia pneumoniae* infection on chromosome 21.**

H.H.H. Göring<sup>1</sup>, J.T. Williams<sup>1</sup>, L. Almasy<sup>1</sup>, M.C. Mahaney<sup>1</sup>, S.A. Cole<sup>1</sup>, M.P. Stern<sup>2</sup>, J.W. MacCluer<sup>1</sup>, J. Blangero<sup>1</sup>. 1) Dept. of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Div. of Clinical Epidemiology, Dept. of Medicine, University of Texas Health Science Center, San Antonio, TX.

For many years, it has been speculated that chronic disease of old age may in part be a result of hidden, long-term infection. The infectious agent(s) may be causing disease directly, or disease may be a consequence of inflammation caused by the host's immune reaction to the pathogen(s). An intriguing candidate is *Chlamydia pneumoniae*, to which accumulating evidence points as potentially being partly responsible for atherosclerosis.

We have conducted a genome-wide linkage screen on seropositivity to *C. pneumoniae* in 591 individuals belonging to 23 large, randomly ascertained pedigrees of Mexican Americans, who are participants in the San Antonio Family Heart Study. Seropositivity was assessed by IgA and IgG ELISA tests on blood serum. Genome-wide variance components-based linkage analysis was conducted using a quantitative liability model for the dichotomous trait seropositivity. The significance was assessed empirically by simulation.

66 % of individuals were found to be seropositive, indicating current infection with *C. pneumoniae*. The additive heritability to infection, after adjusting for sex and age, was estimated as 0.38 +/- 0.14. A (empirical) lod score of 3.2 was obtained on chromosome 21p. No other lod scores over 2 were observed. To our knowledge, this is the first study to demonstrate that sensitivity to *C. pneumoniae* infection is heritable and to localize an underlying genetic factor.

**MULTIDISEQ: Computer software for multipoint linkage analysis of complex traits allowing for marker-marker LD (Linkage Disequilibrium).** *T. Hiekkalinna*<sup>1</sup>, *L. Peltonen*<sup>1,2</sup>, *J.D. Terwilliger*<sup>3,4</sup>. 1) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Department of Medical Genetics, University of Helsinki, Finland; 3) Department of Psychiatry and Columbia Genome Center, Columbia University, NY, USA; 4) New York State Psychiatric Institute, NY, USA.

The current trend in mapping complex traits is use of dense SNP and/or microsatellite marker maps which are in some or complete LD with each other and hypothesized predisposing variant(s). Currently available multipoint linkage analysis software are not well suited for analyzing such marker maps. If the marker-marker LD (i.e., marker-locus haplotype frequencies) is not counted for in multipoint analysis, it results in false positive evidence and significant overestimation of linkage and LD. Consequently it is important to use such multipoint analysis software which can accommodate the marker-marker LD. Here we introduce a new multipoint linkage analysis software MULTIDISEQ, which can accommodate the observed intermarker LD. The program uses a special version of ILINK program of the FASTLINK4.1P package to maximize the likelihood over the marker-marker haplotype frequencies with the presence of LD. Next the program performs the multipoint linkage analysis with maximized haplotype frequencies using the LINKMAP program of the FASTLINK4.1P package. We have applied this method to the actual data of the markers showing tight LD and have been able to exclude false positive results from further analyses. We think this robust method is helpful and user-friendly in current and future linkage studies extracting information from dense set of markers. Software is available from <http://www.helsinki.fi/~tsjuntun/multidiseq/index.html>.

**Fine mapping of susceptibility loci for preeclampsia on chromosomes 2p25, 4q23 and 9p13-p21.** *E. Kerkelä<sup>1</sup>, H. Laivuori<sup>1</sup>, H. Jiao<sup>1</sup>, V.-V. Mäkelä<sup>1</sup>, R. Kaaja<sup>2</sup>, O. Ylikorkala<sup>2</sup>, J. Kere<sup>1</sup>.* 1) Department of Biosciences, Karolinska Institute/Novum, Huddinge, Sweden; 2) Department of Obstetrics and Gynaecology, University of Helsinki, Finland.

Preeclampsia is a common (3%) hypertensive disorder of pregnancy, and one of the leading causes of maternal mortality. It also increases perinatal mortality five-fold. The pathogenesis of this heterogeneous disorder is incompletely understood, but it has a familial component suggesting that one or more common alleles may act as susceptibility genes. We have previously mapped three candidate susceptibility loci for preeclampsia on chromosomes 2p25, 4q32 and 9p13-p21 in 15 Finnish families, recruited predominantly from the Kainuu province (Laivuori et al. *AJHG* 72:168-177, 2003). We increased the marker density in these loci by adding 34 microsatellites at approximately 1 cM intervals. Linkage was assessed by the affecteds-only non-parametric multipoint linkage (NPL) analysis method. We were able to narrow down the candidate area on chromosome 2 with the highest peak showing significant linkage (NPL score 4.09,  $p=0,00036$ ). Instead, NPL scores for two other loci showed only suggestive linkage with no apparent improvement to the previous scan. To study if the overall haplotype distribution in the loci differs among the chromosomes of the affected vs. non-affected individuals, haplotype association analysis using the HPM algorithm (Toivonen et al. *AJHG* 72:168-177, 2003) was done, including testing of significance by permutation tests. Empirically significant associations were observed, supporting and refining considerably the linkage results. We are currently narrowing the associated haplotype further by fine mapping using high-density SNP genotyping.

**Dutch genome-wide scan for preeclampsia revisited.** *A.M.A. Lachmeijer<sup>1</sup>, G. Pals<sup>1</sup>, M. Grippeling<sup>1</sup>, G.A. Dekker<sup>2</sup>, L.P. ten Kate<sup>1</sup>.* 1) Dept Clinical Genetics and Human Genetics, VU University Medical Center, Amsterdam, The Netherlands; 2) Dept Obstetrics and Gynecology, NW Adelaide Health Service, Adelaide, Australia.

Preeclampsia, de novo hypertension and proteinuria in pregnancy, and its more severe form HELLP-syndrome, are among the most common obstetrical disorders and have a familial tendency. Our genome-wide scan in 67 affected sib-pair families (Lachmeijer et al. *EJHG* 2001) showed a maximum lod score of 1.99 on chromosome 12q. The subgroup of preeclamptic families showed suggestive lod scores on 22q and 10q. In the subgroup of HELLP-families the lod score peak on 12q increased to 2.1 whereas it disappeared in the preeclampsia subgroup. Our data did not fit loci found in three previous genome scans from Australia (4q; Harrison et al. *AJHG* 1997), Iceland (2p13; Arngrmsson et al. *Hum Mol Genet* 1999) and Australia/New Zealand (2p,2q; Moses et al. *AJHG* 2000), although two smaller peaks on chromosome 3p and 15q overlapped with peaks in the Icelandic scan. In contrast to our families, the families in the other scans comprised also affected women with a milder phenotype; non-proteinuric hypertension. To assess whether inclusion of the mild phenotype would alter our previous data we performed additional marker analysis at the loci on chromosome 2p13, 2q, 3p, 4q, 10q and 15q in all our 150 sib-pair families comprising 99 women with the mild phenotype. The non-parametric multipoint lod score on 2p13 increased from 0.0058 to 0.87, suggesting a role for this locus in the susceptibility for the mild-phenotype subgroup in part of our families. Lod scores at the other loci did not change substantially. Finding an increase of the lod score of 1.14 to 1.64 at the 10q locus after analysing the data, omitting the families linking to the 2p13 locus, may be another illustration of preeclampsia's presumed genetic heterogeneity.

**A genome-wide scan for the susceptibility loci to cholelithiasis in Chinese population.** *T. Han<sup>1</sup>, W. Yuan<sup>1</sup>, J. Qin<sup>1,2</sup>, J. Fei<sup>2</sup>, Z. Jiang<sup>2</sup>, L. Jin<sup>1,3</sup>, S. Zhang<sup>2</sup>, W. Huang<sup>1</sup>.* 1) Chinese National Human Genome Center at Shanghai, Shanghai, China; 2) Department of Surgery, Ruijin Hospital, Shanghai Second Medical University, Shanghai Institute of Digestive Surgery; 3) Center for Genome Information, Department of Environmental Health, University of Cincinnati.

Cholelithiasis is the presence of gallstones with strong family predisposition among which cholesterol stones are most common. The supersaturation of cholesterol in bile leads to its crystallization and formation of a nidus for stone. The incidence of cholelithiasis in China is about 7%-10%. We collected twelve cholelithiasis pedigrees in Shanghai China including 95 members, among which there are 63 patients with gallbladder stone diseases. We performed a genome-wide scan combined with both parametric and non-parametric linkage analyses for the susceptibility loci to cholelithiasis in these pedigrees. The result indicated that three loci, D3S1266, D9S1682 and D11S902 were shown suggestive evidence for linkage. Non-parametric linkage (NPL) scores at D3S1266 and D9S1682 were 0.94 and 1.92 with P value of 0.17 and 0.04, respectively. The corresponding LOD scores at both loci were 1.35 and 2.07, respectively. A rise of LOD score of D9S1682 from 2.07 to 2.40 was found when we only carried out the linkage analysis in pedigrees with cholelithiasis combined by hypertriglyceridemia. Transmitted disequilibrium test for D11S902 showed a P value of 0.0027. It was found that there was a maximum LOD scores of 1.35 and 2.07 respectively when fine-mapping study was done around both D3S1266 and D9S1682. Therefore, not only the suggestive results from the genome-wide scan were confirmed but also a fine map from 51.4 cM to 8.6 cM for D3S1266 and from 16.9 cM to 5.8 cM for D9S1682 were achieved. It was suggested that genes on chromosome 3, 9 and 11 might be associated with Chinese cholelithiasis pedigrees, and D9S1682 may contain susceptible genes linked to gallbladder stone diseases with hypertriglyceridemia.

**A genome wide scan using 400 non-synonymous SNP markers in DNA pools identifies genes associated with language impairment in children.** *E. Meaburn, L. Schalkwyk, I. Craig, R. Plomin.* SGDP, Institute of Psychiatry, London, United Kingdom.

Language impairment is a common problem of childhood that occurs despite adequate environmental stimulation and in the absence of sensory or neurological deficits. Twin studies have consistently demonstrated a substantial genetic component in its aetiology, which is thought to be due to the combined action of many genes, or quantitative trait loci (QTLs). In order to increase the power to detect genes associated with language impairment, we have used a direct association strategy based on nonsynonymous SNP (nsSNPs) markers, DNA pooling and large sample sizes. Using public databases we identified 400 nsSNPs with common variants in genes that are expressed in the brain. These were then genotyped using SNaPshot primer extension methodology in triplicate DNA pools consisting of 302 language-impaired children and 1000 representative controls. Relative allele frequencies for the case and control pools were compared, and following corrections for variance in the triplicate pools, 24 nsSNPs were significantly different ( $p < .05$ ), when 20 significant differences would be expected on the basis of chance. In order to avoid false positive results, these nsSNPs showing positive associations with language impairment are currently being tested for their association with the entire distribution of general language quantitative trait scores, using DNA sub-pools representing quintiles of the normal distribution derived from the 1000 controls. The QTL theory assumes that an association at the extremes of a quantitative trait will affect the trait throughout the distribution. This constructive replication indicates whether the QTL association replicates just for the extremes or whether the association operates throughout the distribution as predicted by QTL theory. nsSNPs that replicate in the normal distribution will be individually genotyped. The present nsSNP strategy combined with DNA pooling and large samples represents a step towards identifying genes associated with complex traits in the postgenomic era when all functional polymorphisms are known.

**Association analysis of the VR22 locus in late-onset Alzheimer disease.** *E. Martin<sup>1</sup>, P. Bronson<sup>1</sup>, J. Mitchell<sup>1</sup>, D. Schmechel<sup>1</sup>, G. Small<sup>2</sup>, A. Saunders<sup>3</sup>, A. Roses<sup>3</sup>, P-T. Xu<sup>1</sup>, Y-J. Li<sup>1</sup>, J. Vance<sup>1</sup>, J. Haines<sup>4</sup>, J. Gilbert<sup>1</sup>, M. Pericak-Vance<sup>1</sup>.* 1) Duke Univ Medical Center, Durham, NC; 2) UCLA, Los Angeles, CA; 3) GlaxoSmithKline, RTP, NC; 4) Vanderbilt Univ, Nashville, TN.

Regions on chromosome 10q have been linked to both risk and age at onset (AAO) genes in late-onset Alzheimer disease (AD). Younkin et al. (2002) reported association with single nucleotide polymorphisms (SNPs) in the VR22 gene lying in the region of linkage with plasma A42 levels in a small set of families with AD. They also demonstrated association with the AD phenotype in a set of sib pairs with late-onset AD. The VR22 gene codes for the alpha-catenin-like protein, which is believed to be involved in cell adhesion. Association with A42 levels and AD in sibships suggests that this gene may be involved with AD susceptibility. To test this hypothesis, we genotyped two SNPs in a large set of AD families, unrelated cases and age-matched controls. Both family-based and case-control analyses were used to test for association between the SNPs and AD disease status as well as AAO. No association was found with either SNP in the overall set of 276 families (with 936 discordant sib pairs-DSPs) using the pedigree disequilibrium test (PDT) or in the unrelated cases (N=1568) and controls (N=682) using a chi-square test. However, we did find significant association ( $p=0.009$ ) with one SNP using the PDT in the subset of 50 families (with 131 DSPs) having the oldest AAO (mean AAO>75 years). The significance of the association was even greater ( $p=0.007$ ) in families in this subset containing at least one affected individual without an APOE-4 allele (26 families with 81 DSPs). This suggests that the VR22 locus, or one in linkage disequilibrium with this VR22 polymorphism, could explain some risk for AD in families with the latest onset. However, the same association could not be found in the unrelated cases and controls when stratified by AAO and APOE genotype. Nor could we demonstrate association between the SNPs and AAO in the overall samples. Though it is possible that this locus contributes to AD in some families with late-onset AD, the VR22 locus does not appear to have a broad impact on AD risk or AAO.

**Additional support for a Type 2 diabetes susceptibility locus on chromosome 14.** *I. Kovac<sup>1</sup>, R.J. Havlik<sup>2</sup>, D. Foley<sup>2</sup>, R. Peila<sup>2</sup>, A. Singleton<sup>2</sup>, J. Hardy<sup>2</sup>, J. Egan<sup>3</sup>, D. Taub<sup>3</sup>, B. Rodriguez<sup>4</sup>, K. Masaki<sup>4</sup>, D. Curb<sup>4</sup>, W. Fujimoto<sup>5</sup>, A.F. Wilson<sup>1</sup>.* 1) Dept Genometrics, NHGRI, Baltimore, MD; 2) NIA, Bethesda, MD; 3) NIA, Baltimore, MD; 4) Univ. of Hawaii, Honolulu, HI; 5) Univ. of Washington, Seattle, WA.

Type 2 diabetes is a common genetically influenced multifactorial disorder, affecting an estimated 8.6 million Americans over age 60 alone. Our objective was to evaluate candidate regions for potential replication of previous results suggestive of linkage (GENNID study supported by ADA, and scan results provided by GlaxoSmithKline) in an independent sample. The data were derived from a sample of older Japanese Americans with a high prevalence of diabetes living in Hawaii, and included 529 siblings from 175 families. Model-independent sib-pair linkage analyses were performed on markers D14S297, D14S1040, D14S1032, D1S2657, D1S3721 and D1S1616, with SIBPAL (SAGE 4.3). The affected phenotype was either a diagnosis of diabetes or impaired glucose homeostasis; covariates included age and sex. Evidence of decreased proportion of sib-pairs sharing 2 alleles IBD (.19) was observed at the marker D14S297 in 107 discordant sib-pairs ( $p < .02$ ). The test for mean proportion of alleles shared IBD in these 107 sib-pairs (.46) was marginally significant ( $p < .06$ ). Tests of association were performed at this marker only, with FBAT. A chi-square test of the genotypic frequency distributions between affected ( $N=412$ ) and unaffected ( $N=50$ ) subjects was significant ( $p < 0.000014$ ). Because sparseness of the genotypic contingency table can influence chi-square statistics, Monte Carlo simulation for Fisher's exact test (SAS) was also used. The difference in frequency distribution of D14S297 genotypes was significant at the .05 level ( $p \leq .016$ , 99% CI=.013-.019). Absence of allelic association with diabetes, in both FBAT and SAS analyses, may relate to high degree of polymorphism at the marker locus. The present study was limited by the relatively small sample size. Nevertheless, our results suggest additional support for the hypothesis that a susceptibility gene for Type 2 diabetes may reside in a region around the D14S297 marker on chromosome 14.

**Significant linkage on chromosome 15q in recurrent early-onset major depression.** *D.F. Levinson<sup>1</sup>, G.S. Zubenko<sup>2</sup>, R.R. Crowe<sup>3</sup>, J.R. DePaulo<sup>4</sup>, W.S. Scheftner<sup>5</sup>, M.M. Weissman<sup>6,7</sup>, W.N. Zubenko<sup>2</sup>, S. Boutelle<sup>3</sup>, K. Murphy-Eberenz<sup>1</sup>, D. MacKinnon<sup>4</sup>, M.G. Mcinnis<sup>4</sup>, D.H. Marta<sup>5</sup>, P. Adams<sup>6,7</sup>, J.A. Knowles<sup>6,7</sup>, M.G. Alexander<sup>1</sup>, J. Thomas<sup>4</sup>, P. Holmans<sup>8,9</sup>.*  
1) Dept Psychiatry, Univ Pennsylvania, Philadelphia, PA; 2) Dept Psychiatry, Univ Pittsburgh, Pittsburgh, PA; 3) Dept Psychiatry, Univ Iowa, Iowa City, IA; 4) Dept Psychiatry, Johns Hopkins Univ, Baltimore, MD; 5) Dept Psychiatry, Rush-Presbyterian Med Ctr, Chicago, IL; 6) Dept Psychiatry, Coll Physicians & Surgeons, Columbia Univ, NY, NY; 7) NY State Psychiatric Inst, NY, NY; 8) MRC-BSU, Cambridge, UK; 9) Dept Psychological Med, Univ Wales Coll Medicine, Cardiff, UK.

The Genetics of Recurrent Early-Onset Depression (GenRED) project will collect about 650 families (950 independent affected sib pairs) for the NIMH Cell Repository by 9/03. Preliminary genome scan results are presented for the first 297 families. Proband had recurrent major depressive disorder (MDD-R) with onset before age 31, with at least one sibling (and sometimes additional relatives) with MDD-R and onset before 41. The Center for Inherited Disease Research genotyped 392 microsatellite markers in 1,057 individuals, including 819 affected cases (415 independent ASPs, 660 total affected pairs). Multipoint allele-sharing analysis of all affected pairs was carried out using ALLEGRO (exponential model), and P-values computed by simulation. Preliminary analyses demonstrate genome-wide significant linkage on chromosome 15q ( $Z_{lr} = 4.14$ , empirical genome-wide  $P=0.016$ ). No other locus produced significant or suggestive evidence for linkage. Power would be >80% to detect significant linkage for a locus associated with population-wide  $sibs$  (RR) of about 1.8 in this preliminary sample, or 1.3 for the full sample. Secondary logistic regression analyses considered the effects of covariates including sex (MM, MF, FF pairs), age at onset (AAO), comorbid substance use and anxiety disorders. Accounting for sex increased the evidence for linkage on chromosome 15q, and also on three chromosomes without genome-wide significant linkage (6, 8 and 17); accounting for AAO increased the evidence for linkage on chromosomes 8 and 18.

**Linkage analyses of myostatin pathway genes in human muscle mass: the Leuven Genes for Muscular Strength project.** *M.A. Thomis<sup>1</sup>, W. Huygens<sup>1</sup>, R. Janssen<sup>2</sup>, M. Peeters<sup>1</sup>, R. Vlietinck<sup>2,3</sup>, G. Beunen<sup>1</sup>.* 1) Fac. Physical Education and Physiotherapy, Katholieke Univ. Leuven, Leuven, Belgium; 2) Division of Genetics and Molecular Cell Biology, University Maastricht, The Netherlands; 3) Center for Human Genetics, Faculty of Medicine, Katholieke Univ. Leuven, Belgium.

Myostatin (GDF8) is a transforming growth factor- family member that clearly acts as a negative regulator of skeletal muscle mass in animals. This preliminary study investigated evidence for linkage of 11 microsatellite markers near or within 11 candidate genes of the myostatin pathway in humans as putative QTLs for variation in human muscle mass. Muscle mass indicators (circumferences, estimated muscle+bone cross-sectional areas and volumes) and body composition (fat and fat-free mass) were assessed in 748 male Caucasian sibs from 335 families in the Leuven Genes for Muscular Strength project. Upper-limit heritabilities ranged from 0.57 in adipose tissue measures to 0.90 for several muscle mass indicators. Single-point linkage analysis was performed using Merlin software (Abecasis et al., *Nat Genet* 30:97-101, 2002) and combined identity by descent allele sharing and phenotypic information was analyzed using the implemented regression-based QTL-analysis approach (Sham et al., *Am J Hum Genet* 71:238-253, 2002) as well as variance component analysis in a subset of 108 paired observations. Four phenotypes showed suggestive evidence for linkage with D2S118 (GDF8). Highest LOD scores (1.176,  $P=0.01$ ) were found for muscle+bone area of the mid-thigh and muscle area of the quadriceps. Percent body fat and fat free mass showed less pronounced LOD scores (0.76,  $P=0.03$  and 0.61,  $P=0.05$ ). Suggestive signals for linkage were observed for percentage body fat and sum of 10 subcutaneous skinfolds with markers D12S326, D12S1708 (MYF5, MYF6: 0.68LOD1.6) and to a lesser extent with D12S1030 (IGF-1). Multi-point QTL linkage analyses results on a larger set of siblings will be reported.

**MCMC analysis of complex traits caused by multiallelic loci.** *E.A. Rosenthal*<sup>1</sup>, *E.M. Wijsman*<sup>1,2</sup>. 1) Department of Biostatistics, University of Washington, Seattle, WA; 2) Division of Medical Genetics, University of Washington, Seattle, WA.

Complex traits are affected by multiple loci. While analytic methods used in the analysis of complex traits are often based on a diallelic trait model, many traits are known or suspected to be multiallelic. For example, APOE, a triallelic locus, plays a role in Alzheimer's disease and cardiovascular disease. Multiallelism is problematic when alleles are common, in which case more than one risk allele may be segregating in a family. Analyses of complex traits are further complicated by the lack of information concerning the number of underlying loci and the number of alleles at each trait locus. In order to study common complex traits, linkage analyses may need to allow for multiple underlying loci each with multiple alleles. Loki is a program that uses reversible jump MCMC, which allows the number of quantitative trait loci (QTL) to be a variable in a multipoint linkage analysis of a quantitative trait. However, an underlying assumption is that each QTL is diallelic. This assumption may reduce the ability to detect and localize underlying multiallelic QTL. We test Loki's performance when a trait is caused by a triallelic locus. Traits are simulated using a real data set consisting of multilocus marker data from chromosome 19 on four large pedigrees, ranging in size from 48 to 87 individuals. By grouping marker alleles for one locus into a three allele system and simulating quantitative traits based on this system, we can incorporate the complexities of multilocus transmission inherent in real data while maintaining investigative control over the trait model. Analysis results vary depending on the amount of dominance and heritability ( $h^2$ ) of the linked trait locus. For example, as  $h^2$  increases from 0.4 to 0.9, the localization bias decreases from 38.3 to 3.7 cM in a purely additive model. In the case of dominance, when the broad sense heritability ( $H^2$ ) ranges from 0.2 ( $h^2 = .2$ ) to 0.8 ( $h^2 = 0.7$ ), the localization bias decreases from 9.7 to 7.7 cM. Furthermore, in some cases, Loki appears to compensate for the diallelic assumption by fitting two or more loci near the true location. Potential remedies will be discussed. Supported by NIH HG00035 and GM46255.

**A genome-wide scan for age-related macular degeneration susceptibility loci.** *Y. Zhao*<sup>1</sup>, *BM. Yashar*<sup>2,3</sup>, *GR. Goncalo*<sup>1</sup>, *S. Zarepari*<sup>2</sup>, *E. Trager*<sup>2</sup>, *K. Branham*<sup>2</sup>, *A. Reddick*<sup>2</sup>, *J. Richards*<sup>2</sup>, *A. Swaroop*<sup>2,3</sup>, *AMD Clinical Study Group*. 1) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 2) Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI; 3) Department of Human Genetics, University of Michigan, Ann Arbor, MI.

Age related macular degeneration (AMD), one of the leading causes of irreversible vision loss among the elderly, is a clinically complex disease that is characterized by a variety of retinal pathologies. Family history is one of the strongest risk factors for the development of AMD, underscoring the importance of genetic loci in the development of disease. Here we report the results of a genome-wide search for these loci. We examined 781 microsatellite markers (average spacing 4.7, standard deviation 2.6 cM) from Marshfield Clinic in 333 affected individuals and 93 unaffected relatives in 116 families. Probands were identified from ophthalmic clinics in the U-M Health Care System. Retinal findings in all participants were evaluated in accordance with the AMD International Grading System. The average age of diagnosis for affected individuals was 70.4 years, and slightly lower at 68.8 years for individuals with geographic atrophy (GA). Disease in probands was biased towards terminal forms; greater than 80% were affected with either GA or neovascular AMD. We performed an affected relative pair analysis using non-parametric methods. We identified 2 loci linked to geographic atrophy on chromosome 1 (NPL LOD score 2.01) and chromosome 5 (NPL LOD score 2.65) and three loci linked to neovascular disease on chromosome 2 (NPL LOD score 1.96), chromosome 9 (NPL LOD score 1.64) and chromosome 22 (NPL LOD score 1.87). Two of these loci also showed substantial evidence for linkage among all AMD cases. Our results are in good agreement with previous studies, support the importance of genetic risk factors in the development of disease and suggest that distinct genetic pathways influence variations in clinical manifestations.

**A genome-wide screen for autism susceptibility loci identifies significant linkage on 19p.** *J.S. Sutcliffe<sup>1</sup>, L.M. Olson<sup>1</sup>, J.L. McCauley<sup>1</sup>, M. Dowd<sup>2</sup>, B. Winkloski<sup>2</sup>, B. Rosen-Sheidley<sup>2</sup>, G. Crockett<sup>1</sup>, S.E. Folstein<sup>2</sup>, J.L. Haines<sup>1</sup>.* 1) Program in Human Genetics, Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN; 2) Department of Psychiatry, Tufts University-New England Medical Center, Boston, MA.

Autism is a complex genetic neuropsychiatric condition characterized by deficits in social interaction and language and patterns of repetitive or stereotyped behaviors and restricted interests. Several genomic screens in autism families have been performed, and these have identified loci on 7q and 2q as being consistently replicated in most studies. We report a second-generation, 10 cM genomic screen of multiplex autism families, including 81 families recruited from New England and 80 well-characterized families from the AGRE collection. Two-point and multipoint heterogeneity LOD (HLOD) scores were calculated for both dominant and recessive models, and nonparametric allele-sharing was calculated using Allegro. One locus mapping to ~40 cM on chromosome 19p achieved genome-wide significance, with a multipoint HLOD of >3. Other suggestive signals (HLODs > 1.6) were detected on 17q, 6q, 16p and 12p, and of these, all but the 12p site represents a replication of suggestive linkage identified by other autism genomic screens. To homogenize our dataset for these studies and to relate particular loci to phenotypic domains in autism, we subdivided our families according to genetically-relevant ADI-based subsets. While no appreciable effect was evident on the 19p locus, other regions showed significantly increased linkage in specific subsets, supporting the utility of this approach. These results highlight the locus and phenotypic heterogeneity in autism and point to chromosomal positions likely to harbor autism susceptibility genes.

**A genome-wide scan in familial ankylosing spondylitis susceptibility.** *J.D. Reveille<sup>1</sup>, J. Bruckel<sup>2</sup>, R. Inman<sup>3</sup>, M. Mahowald<sup>4</sup>, M. Weisman<sup>5</sup>, H.R. Schumacher<sup>6</sup>, M.A. Khan<sup>7</sup>, W.P. Maksymowych<sup>8</sup>, D.T.Y. Yu<sup>5</sup>, M. Stone<sup>3</sup>, J. Lee<sup>9</sup>, L. Jin<sup>9</sup>.* 1) Division of Rheumatology, University of Texas - Houston, Houston, TX; 2) Spondylitis Association of America, Sherman Oaks, CA; 3) University of Toronto, Toronto, ON; 4) University of Minnesota, Minneapolis, MN; 5) University of California-Los Angeles, Los Angeles, CA; 6) University of Pennsylvania, Philadelphia, PA; 7) Case-Western Reserve, Cleveland, OH; 8) University of Alberta, Edmonton, AB, Canada; 9) Center for Genome Information, University of Cincinnati, Cincinnati, OH.

Although the MHC is likely the primary mediator of genetic susceptibility to ankylosing spondylitis (AS), it accounts for less than 50% of the total genetic risk. In order to define the genetic basis of susceptibility to AS, 269 affected sibpairs of primarily European ancestry concordant for AS by modified New York criteria with available sacroiliac radiographs from 121 pedigrees were genotyped for 400 markers in ABI PRISM Linkage Map. Two point and multi-point non-parametric linkage analyses were conducted using the NPL statistic. The linkage of MHC region was supported by both two-point and multi-point analyses with the strongest peak at 44.41 cM (D6S276,  $p = 3.5 \times 10^{-4}$ ) and at 48.37 cM ( $p = 7.3 \times 10^{-10}$ ), respectively. A second region was found to have positive linkage at the q arm of chromosome 6 (D6S441) in two-point analysis supported by a 29 cM region (148.47-177.33 cM) in multi-point analysis with the smallest  $p$  ( $3.0 \times 10^{-3}$ ) at 162.25 cM. Another broad region was found on chromosome 11q positively linked to AS separated by two single markers (D11S908 and D11S4151) supported by multi-point analysis spanning 68 cM continuously from 88.89 cM to 156.86 cM with the strongest peak at 125.84 cM ( $p = 1.2 \times 10^{-3}$ ). Weaker evidence of linkage was found on chromosome 1q, as well as chromosomes 5q and 19q, the latter two having been also seen in another scan (Laval et al. 2001). Thus this genome-wide scan implicates, in addition to the MHC, regions outside the MHC in AS susceptibility, especially on chromosomes 6q and 11q.

**A genome-wide linkage scan for QTL influencing the skeletal maturation of healthy children in the Fels Longitudinal Study.** *B. Towne<sup>1</sup>, J. Blangero<sup>2</sup>, J.S. Parks<sup>3</sup>, T. Dyer<sup>2</sup>, S.A. Cole<sup>2</sup>, M.R. Brown<sup>3</sup>, D.L. Duren<sup>1</sup>, A.F. Roche<sup>1</sup>, R.M. Siervogel<sup>1</sup>.* 1) Wright State University School of Medicine, Dayton, OH; 2) Southwest Foundation for Biomedical Research, San Antonio, TX; 3) Emory University School of Medicine, Atlanta, GA.

Most current knowledge of genetic influences on skeletal development comes from studies of monogenic disorders. Few studies have examined genetic influences on normal variation in skeletal development. During childhood, the skeletal age (SA) of a normal child can vary up to 3 years from that child's chronological age. In this study, we present initial findings from linkage analyses of the SA of children assessed at chronological ages 1 to 17 years. SA data were obtained from 9,865 hand-wrist radiographs taken between 1931 and 2002 of 1,069 children from 220 families in the Fels Longitudinal Study (Roche, 1991). Estimates of SA were made using the FELS method (Roche et al., 1988), a maximum likelihood approach that uses measured and graded indicators of skeletal development appropriate for a given chronological age. An initial set of 478 subjects was genotyped for 377 autosomal markers spaced approximately every 10 cM. A variance components-based linkage analysis method (SOLAR; Almasy and Blangero, 1998) was used to obtain multipoint LOD scores. Significant LOD scores ( $> 3.0$ ) were found for SA during middle childhood - a LOD of 3.37 for linkage of SA at chronological age 8 to a QTL on chromosome 8 at 122 cM (between markers D8S270 and D8S1784), and a LOD of 3.40 for linkage of SA at chronological age 10 to a QTL on chromosome 3 at 44 cM (between markers D3S2338 and D3S1266). Nine suggestive LOD scores ( $> 1.9$ ) were found for SA at chronological ages 1, 2, 4, 7, 11, and 13 years. These results indicate the existence of genes on chromosomes 3 and 8 that influence the tempo of normal skeletal maturation during middle childhood, and suggest other genes that influence skeletal maturation at other childhood ages. Future work will seek to identify specific genes that influence particular aspects of skeletal maturation at different stages of childhood development. Supported by NIH grants R01HD36342, R01HD12252, and R01MH59490.

**A new locus for disseminated superficial actinic porokeratosis at Chromosome 17q24-25.1.** Z. Zhang<sup>1,2</sup>, W. Yuan<sup>1</sup>, J. Zhang<sup>1</sup>, F. Jiang<sup>2</sup>, Z. Niu<sup>1</sup>, J. Zhao<sup>2</sup>, W. Liu<sup>2</sup>, W. Huang<sup>1</sup>. 1) Chinese National Human Genome Center at Shanghai, Shanghai, China; 2) Institute of Dermatology, Chinese Academy of Medical Sciences, Peking Union Medical College, Nanjing, China.

Disseminated superficial actinic porokeratosis (DSAP) is an autosomal dominant localized skin keratinization disorder, characterized by multiple superficial keratotic lesions surrounded by a slightly raised keratotic border. The susceptible loci for DSAP have been previously mapped to chromosomes 12q23.2-24.1 and 15q25.1-26.1, respectively. However, no gene for DSAP has been identified and the genetic basis and pathogenesis for this disorder are still unclear to date. To identify the causative gene in a four-generation Chinese DSAP family, we initially performed linkage analysis with microsatellite markers located on 12q23.2-24.1 and 15q25.1-26.1 and failed to confirm the linkage to these regions. Therefore, a genome wide scan with 382 microsatellite markers from the autosomes was performed and two-point linkage analysis of the members in this family showed supportive lod score for markers D17S949 ( $Z_{\max} = 2.30$ , at  $\alpha = 0.00$ ). The fine mapping with 8 polymorphic microsatellite markers between D17S944 and D17S785 were further tested and the haplotype analysis showed that the susceptible gene for DSAP in this family lies in an 13.0-cM interval between D17S944 and D17S1351. Our data suggest DSAP is a genetic heterogeneous disorder. It is conceivable that the underlying genes for 12q23.2-24.1, 15q25.1-26.1 and 17q24-25.1 loci are involved in the same biochemical pathway.

**Identification of the cylindromatosis tumor-suppressor gene responsible for multiple familial trichoepithelioma.**

*X. Zhang<sup>1</sup>, Y. Liang<sup>1,2</sup>, P. He<sup>1,2</sup>, S. Yang<sup>1</sup>, Y. Shen<sup>2</sup>, Y. Yuan<sup>2</sup>, Y. Cui<sup>1</sup>, W. Huang<sup>2</sup>.* 1) Institute of Dermatology, Anhui Medical University, Hefei, Anhui, China; 2) Chinese National Human Genome Center at Shanghai, Shanghai, China.

Multiple familial trichoepithelioma is an autosomal dominant skin disease characterized by the presence of many small tumors predominantly on the face. The first locus has been previously mapped to chromosome 9p21, but no gene for multiple familial trichoepithelioma has been identified to date. To identify the causative gene in a large Chinese family, we initially performed linkage analysis with microsatellite markers located on 9p21 and failed to confirm the linkage to this region. Previous publications showed multiple familial trichoepithelioma and hereditary cylindromatosis could occur within one family and in a single person. Therefore we speculated the CYLD1 gene is responsible for hereditary cylindromatosis, which may be related with the pathogenesis of multiple familial trichoepithelioma. Consequently, we genotyped all available individuals using 11 microsatellite markers spanning the CYLD1 gene at 16q12-13. We identified the linkage of multiple familial trichoepithelioma to this region. Through mutation analysis in the CYLD1 gene by sequencing, we detected a frameshift mutation, designated 5-8 delCAGA in the exon 18. The study firstly identified the CYLD1 gene responsible for multiple familial trichoepithelioma and showed that different mutations of the CYLD1 gene can give rise to distinct clinical and histological expression such as hereditary cylindromatosis and multiple familial trichoepithelioma.

**A genome-wide linkage scan for Photoparoxysmal Response.** *D. Pinto*<sup>1,2</sup>, *B. Westland*<sup>2</sup>, *G-J. de Haan*<sup>1</sup>, *J. Parra*<sup>1</sup>, *D.G. Kasteleijn-Nolst-Trenite*<sup>1</sup>, *G. Rudolf*<sup>1</sup>, *B. Neubauer*<sup>1</sup>, *U. Stephani*<sup>1</sup>, *A.M. da Silva*<sup>1</sup>, *B. Pedersen*<sup>1</sup>, *D. Parain*<sup>1</sup>, *H. Stroink*<sup>1</sup>, *L. Lagae*<sup>1</sup>, *M. Piccioli*<sup>1</sup>, *M. Brinciotti*<sup>1</sup>, *M.L. Friis*<sup>1</sup>, *P. Bonnani*<sup>1</sup>, *T. Covanis*<sup>1</sup>, *D. Lindhout*<sup>1,2</sup>, *B.P.C. Koeleman*<sup>1,2</sup>. 1) European Consortium on Genetic Analysis of Photosensitivity and Visual Sensitive Epilepsies; 2) Department of Medical Genetics, Univ Medical Center Utrecht, Utrecht, Netherlands.

Photosensitivity is defined as the occurrence of a photoparoxysmal response (PPR) on the EEG during intermittent photic stimulation. PPR is a major finding across several Idiopathic Generalized Epilepsies (IGEs), especially Juvenile Myoclonic Epilepsy (JME). Segregation studies suggest that PPR is an autosomal dominant trait with age-related reduced penetrance, in which case standard linkage analysis is expected to have relative high power to detect the underlying disease locus. In order to identify the susceptibility locus for PPR, a European Consortium has been formed which aims at collecting PPR-families and performing a joined genome-wide linkage scan. Through this collaborative action we have collected 39 multiplex pedigrees from Dutch, French and German origin, ascertained by the presence of 2 or more individuals with photosensitivity in the EEG. All patients were diagnosed by standardized IPS protocol and classification system. A first-stage genome scan was done using 427 microsatellite markers with inter-marker distance of <10 cM in which 274 family members were genotyped. Parametric and Non-Parametric multipoint Linkage (NPL) analyses using the Genehunter program were performed. NPL analysis provided four interesting chromosomal regions that met our initial criteria of a P value < 0.05. These promising regions are currently being followed-up. Identification of the PPR gene(s) will help resolving the genetic background of the common IGEs and may clarify the effect of the PPR gene in the polygenic background of JME.

**Genome-Wide Search for QTL traits of Schizophrenia Reveals a Locus for Verbal Learning and Memory on 4q and for Visual Working Memory on 2q.** *T. Paunio*<sup>1, 2</sup>, *AM. Tuulio-Henriksson*<sup>1</sup>, *T. Hiekkalinna*<sup>1</sup>, *M. Perola*<sup>1</sup>, *T. Varilo*<sup>1</sup>, *T. Partonen*<sup>1</sup>, *T. Cannon*<sup>3</sup>, *J. Lonngqvist*<sup>1</sup>, *L. Peltonen*<sup>1, 3</sup>. 1) National Public Health Institute, Finland; 2) Helsinki University Hospital, Finland; 3) UCLA, USA.

In the light of the current linkage studies and numerous tentatively identified associated loci we can conclude that genetic background of schizophrenia seems extremely heterogeneous. Further, the clinical end state diagnosis most probably does not provide the ideal phenotype for the identification of involved genes. In our population-wide study sample of Finnish schizophrenia families the best evidence for linkage, using dichotomized phenotype classification, has been obtained for markers on 1q and 5q (Paunio et al. 2001). Here we have used a set of quantifiable variables derived from neuropsychological test data in a genome-wide variance component-based linkage analysis (SOLAR) in 168 Finnish families ascertained for schizophrenia. All the analyzed QTLs are closely associated with vulnerability to the disorder. Our major findings were a locus for functions related to verbal learning and memory on 4q, for visual working memory on 2q and for recognition memory on 10p. Many variables measuring verbal learning and memory showed linkage to 4q. The best individual two-point lod score was obtained for long-term memory on 4q ( $Z = 3.01$ ). Among the measures assessing attention and working memory, the best evidence of linkage was obtained for visual working memory on 2q ( $Z = 2.80$ ). For recognition memory the best two point lod score was obtained for 10p ( $Z = 2.27$ ). Interestingly, when families were ascertained for the linkage of schizophrenia to either 1q or 5q, the evidence of linkage of long-term memory to 4q increased in those families not linked to 5q ( $Z = 4.87$ ). In contrast, this did not occur with linkage of visual working memory to 2q. This provides some initial evidence of negative interaction between the schizophrenia locus on 5q31 and the QT locus on 4q. The results confirm and extend previous linkage findings on 4q and 2q and demonstrate the benefits of using measurable quantitative trait components, instead of clinical diagnosis, in search for genes involved in susceptibility to schizophrenia.

**Linkage analyses using autoantibody traits as intermediate phenotypes in lupus pedigrees.** *P.S. Ramos<sup>1</sup>, J.A. Kelly<sup>2</sup>, C.M. Meyer<sup>1</sup>, A.N. Leiran<sup>1</sup>, W.A. Ortmann<sup>1</sup>, K.J. Espe<sup>1</sup>, G.R. Bruner<sup>2</sup>, J.M. Olson<sup>3</sup>, J.B. Harley<sup>2</sup>, K.L. Moser<sup>1</sup>.*  
1) Dept Medicine, Univ Minnesota, Minneapolis, MN; 2) Oklahoma Medical Research Foundation, Oklahoma City, OK; 3) Case Western Reserve University, Cleveland, OH.

Numerous studies have suggested that various autoimmune phenotypes may share underlying genetic components. Given the higher incidence of autoimmune diseases and serologic abnormalities in family members of systemic lupus erythematosus (SLE) probands, we hypothesized that redefining phenotypes of individuals in SLE pedigrees with a propensity for autoimmunity may provide a useful intermediate phenotype for unraveling the complexity of SLE and possibly other autoimmune diseases. We have characterized lupus-related autoantibody profiles of 1668 total subjects in 229 families multiplex for SLE. Based on the presence of specific autoantibodies commonly associated with SLE we have defined a novel phenotype for lupus-related autoimmunity (LRA). We also used measures of association between siblings and familial correlations for each autoantibody to determine which specificities were most likely to aggregate in families and provide potentially useful information for subsequent linkage analyses. We identified evidence of familial aggregation for La/SSB ( $p=0.001$ ,  $OR=4.4$ ), nRNP ( $p=0.009$ ,  $OR=2.5$ ), and Sm ( $p=0.04$ ,  $OR=3.0$ ). Genome wide linkage analyses were performed using both the revised Haseman-Elston algorithm (SIBPAL) and an affected relative pairs approach (LODPAL) to identify chromosomal regions of increased allele sharing. Significant evidence for linkage ( $p<0.00005$ ) was found using La/SSB autoantibodies as the trait for 8 loci: 6p23, 10q23, 11p15, 12q24, 15q22, 16q23, 17q25, and 19p13. Several additional loci with suggestive evidence for linkage ( $p<0.002$ ) were identified using various other autoantibody traits. These results demonstrate that using autoantibody traits as intermediate phenotypes may increase the power to detect linkage, and provide evidence of the presence and locations of genes that are involved in the development of lupus-related humoral autoimmunity. Identification of these genes is likely to help further unravel the complexity of SLE as well as other autoimmune diseases.

**Linkage of biochemical and molecular traits related to obesity in the Old-Order Amish.** *G.J. Papanicolaou<sup>1</sup>, P. Platte<sup>2</sup>, E.W. Pugh<sup>3</sup>, K. Doheny<sup>3</sup>, K.M. Pirke<sup>4</sup>, M.-H. Roy-Gagnon<sup>1,5</sup>, A.J. Stunkard<sup>6</sup>, C. Francomano<sup>7</sup>, A.F. Wilson<sup>1</sup>.*  
1) Genometrics Section, NIH/NHGRI/IDRB, Baltimore, MD; 2) Biological & Clinical Psych., U. Wuerzburg, Germany; 3) CIDR, JHU SOM, Balt., MD; 4) Ctr. Psychobiology & Psychosomatic Res., Univ. Trier, Germany; 5) Dept. Epidemiology, JHSPH, Balt., MD; 6) Dept. Psychiatry, U. PA, Phila., PA; 7) NIA, NIH, Balt., MD.

The gradual increase in overweight in the U.S., first observed in the 1960s, has increased significantly in the past decade; today nearly two-thirds of the population is overweight and over thirty percent are obese. As part of an ongoing study of traits related to obesity in the Old-Order Amish, seven two- and three- generation families totaling 157 individuals (with a mean sibship of 7.2) were assessed for 21 obesity related traits, including diastolic and systolic blood pressure, and levels of guanine nucleotide-binding protein, beta-3 (GNB3), glucose, insulin, leptin, triiodothyronine (t3), thyroxin (t4), thyroid stimulating hormone (tsh), and uncoupling protein 3 (UCP3). Although families were ascertained on a surrogate measure of obesity, the families were normotensive and did not exhibit signs of diabetes. Genotyping was performed at the Center for Inherited Disease Research (Baltimore, MD) with a modified Marshfield Genetics 8 marker set consisting of 384 short tandem repeat markers with an average distance of 9 cM. Model-independent linkage analysis identified candidate regions for diastolic b.p., glucose, insulin, leptin, t3, t4, and tsh. Traits GNB3 and UCP3 were mapped to respective known locations, providing both an internal control for the analysis as well as an estimation of Type I error.

**A genome-wide scan for age-related macular degeneration provides evidence for linkage to several chromosomal regions, including significant linkage to chromosomes 2, 3, 6, and 8.** *S.L. Santangelo*<sup>1,2,3</sup>, *K. Book*<sup>4</sup>, *C-H. Yen*<sup>1</sup>, *J.M. Seddon*<sup>2,3,4</sup>. 1) Psychiatric and Neurodevelopmental Genetics Unit, Massachusetts General Hospital, Charlestown, MA; 2) Harvard Medical School, Boston, MA; 3) Harvard School of Public Health, Boston, MA; 4) Ophthalmology/Epidemiology, Massachusetts Eye and Ear Infirmary, Boston, MA.

We report the results of a genome-wide linkage scan for age-related macular degeneration (AMD) in 158 multiplex families. AMD classification was based on fundus photography and was assigned a grade ranging from 1 (no disease) to 5 (exudative disease) according to a modified grading system used in the Age-Related Eye Disease Study (AREDS Research group, 2001). Genotyping was performed by the NHLBI Mammalian Genotyping Service at Marshfield (404 short tandem repeat markers, Weber Screening Set 10). The sample included 158 families with 2 or more siblings with AMD, 490 affected individuals (grade 3 or higher), 101 unaffected (grades 1 or 2 over age 60), and 38 whose affection status was unknown (grades 1 or 2, under age 60). Relative pairs included 511 affected sibling, 28 avuncular, 53 cousin, 7 grandparent-grandchild, and 9 grand avuncular pairs. Two-point parametric and multipoint parametric and nonparametric analyses were performed.

Maximum 2-point LOD scores ranging from 1.0-2.0 were found for markers on chromosomes 1,2,8,10,14,15 and 22. Multipoint analyses were consistent with 2-point results for chromosomes 1,2,8,10 and 22 and provided evidence for additional linkage regions on chromosomes 3,6,8,12,16 and X. Our signals on chromosomes 1q, 6p, and 10q are consistent with previously published results (e.g., Klein et al. 1998; Weeks et al. 2000; 2001; Schick et al. 2003). Our strongest evidence for linkage was found on chromosomes 2 (1 marker), 3 (2 adjacent markers), 6 (2 adjacent markers), and 8 (7 contiguous markers); these multipoint signals were statistically significant, with empirical p-values = 0.00001. However, we identified several additional regions on chromosomes 1,10,12,16, and 22 that warrant further study because of internally consistent evidence and/or consistency with other published AMD scans.

**Linkage and association studies of the controversial locus 6p21 in idiopathic generalized epilepsies (IGE).** P.A.O. Ribeiro<sup>1</sup>, V.D.B. Pascoal<sup>1</sup>, L.E. Betting<sup>2</sup>, N.F. Santos<sup>1</sup>, R. Secolin<sup>1</sup>, S.B. Mory<sup>2</sup>, C.A.M. Guerreiro<sup>2</sup>, M.M. Guerreiro<sup>2</sup>, F. Cendes<sup>2</sup>, I. Lopes-Cendes<sup>1</sup>. 1) Department of Medical Genetics, UNICAMP, Campinas, Brazil; 2) Department of Neurology, UNICAMP, Campinas, Brazil.

**Purpose:** The first *locus* for IGE was mapped on chromosome (ch) 6p21 (the HLA region), in families segregating juvenile myoclonic epilepsy (JME), a common type of IGE. Additional linkage and association studies were performed in many IGE families leading to conflicting results. One hypothesis to explain these is the presence of genetic heterogeneity, which may be related to differences in ethnic origin of the families studied. The aim of the present study was to investigate linkage and/or association between the gene(s) predisposing to IGE and the candidate *locus* on ch 6p21 in Brazilian patients. **Methods:** We performed linkage analysis of 6 unrelated families, with at least 2 IGE cases. For the association study we analyzed 44 unrelated patients with IGE and 54 normal controls. For both studies we genotyped 3 microsatellite markers flanking the HLA region on ch 6p21: tel. D6S276, D6S265 and D6S291 cent. Two-point and multipoint lod-scores were calculated using the software LINKAGE. For the association studies we used chi-square and Fishers exact test. **Results:** Linkage simulation analysis of the 6 families combined yield a  $Z_{max} = 5.09$ . Lod-scores were negative for all 3 markers genotyped (for = 0: D6S276,  $Z = -7.22$ ; D6S265,  $Z = -4.16$ ; D6S291,  $Z = -1.23$ ), excluding the entire HLA region. There was no significant association between any allele of 2 microsatellites genotyped (D6S276 and D6S265,  $p$  ranging between 0,09 and 1,0). However, allele 1 of the D6S291 marker occurred more frequently in controls as compared to patients ( $p = 0,04$ ). **Conclusion:** Linkage results excluded the presence of a major *locus* predisposing to IGE in ch 6p21. However, the association study indicate the presence of a modifier *locus*, that could be involved in protection against IGE. These results confirm the hypothesis of genetic heterogeneity among IGE and points to the presence of complex inheritance in this disorder.

Supported by CAPES and FAPESP.

**Haplotype associations between ADAM33 and childhood asthma.** *B. Raby*<sup>1</sup>, *E.K. Silverman*<sup>1</sup>, *C. Lange*<sup>2</sup>, *R. Lazarus*<sup>1</sup>, *D.J. Kwiatkowski*<sup>3,4</sup>, *S.T. Weiss*<sup>1,4</sup>. 1) Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 2) Department of Biostatistics, Harvard School of Public Health, Boston, MA; 3) Hematology Division, Brigham and Womens Hospital, Boston, MA; 4) Harvard Partners Center for Genetics and Genomics, Boston, MA.

ADAM33 (chr.20p13) has been implicated as an asthma-susceptibility gene using a positional cloning approach (Nature 2002). However, genetic linkage of asthma phenotypes to chr.20p13 has not been observed in most asthma genome scans, and it is unclear whether these associations with ADAM33 are broadly generalizable. We performed a family-based association study of ADAM33 using parent-child trios ascertained through the Childhood Asthma Management Program (CAMP). CAMP is a multi-centered, randomized placebo-controlled clinical trial comparing the efficacy of long-term inhaled anti-inflammatory medication use in improvement of lung function. Of the 1041 children enrolled in the clinical trial, DNA was available for 968, as well as 1518 of their parents. In total, 652 parent-child trios were available for analysis. 17 SNPs (including 9 associated with asthma in the initial report) were genotyped using the Sequenom mass spectrometry platform. All 17 SNPs were in Hardy-Weinberg Equilibrium among the parents. Among Caucasians, 11 haplotypes with frequency of at least 1% were observed. Single-SNP and haplotype association analysis was performed using the FBAT program for both the asthma diagnosis phenotype and four quantitative phenotypes (percent predicted forced expiratory volume in one second, airways responsiveness, total serum immunoglobulin E levels [IgE], and total serum eosinophil counts). No single-SNP association was observed, regardless of genetic model specification. However, a common haplotype (frequency 14.6%) was associated with asthma ( $p=0.03$ ), FEV1 ( $p=0.04$ ), IgE ( $p=0.07$ ), and serum eosinophils ( $p=0.02$ ). These data provide support for an asthma locus in the ADAM33 genomic region. However, the magnitudes of the observed associations are modest, and suggest that either the gene has only modest effects or that the true asthma locus is located near, but not at, ADAM33. Further SNP mapping of this region is needed.

**Linkage of Speech Sound Disorder to Dyslexia candidate regions.** *S.D. Smith*<sup>1</sup>, *K.E. Deffenbacher*<sup>1</sup>, *R. Boada*<sup>2</sup>, *N. Raitano*<sup>2</sup>, *R. Tunick*<sup>2</sup>, *B.F. Pennington*<sup>2</sup>. 1) Pediatrics, Univ Nebraska Med Ctr, Omaha, NE; 2) Psychology, Univ. Denver, Denver, CO.

Speech Sound Disorder (SSD) is a developmental disability in learning to produce intelligible speech, noted in the preschool years. In contrast, specific reading disability (dyslexia) is diagnosed at school age and involves difficulty learning to read and spell. SSD has been conceptualized as primarily a motor disorder, while in the majority of cases the core deficit of dyslexia is in the ability to recognize phonemes of language and map them on to letters. Despite these apparent differences, it has been observed that SSD and dyslexia cosegregate, suggesting that they may have common cognitive and genetic etiologies. Sixty-nine families were identified with at least one child between the ages of 5-6 years with a history of moderate to severe SSD, and one sib between the ages of 5-8 years who could be evaluated. Five phenotypes were used, 4 quantitative variables assessing speech sound and phonologic abilities and one categorical phenotype that assigned affection status based on clinical history. Sib-pair linkage analysis was done with markers from 3 chromosomal regions previously linked with dyslexia: 1p36, 6p21.3, and 15q21. Single point analysis was done with SIBPAL (SAGE 4.4), with GENEHUNTER2 and QMS2 used for multipoint analysis. All three regions showed some indication of linkage with SSD phenotypes. The strongest results were for single point analysis of the categorical phenotype and 6p21.3, with highest significance at D6S1571 ( $p=0.00051$ ). Multipoint analyses (QMS2) were suggestive for 3 of the quantitative phenotypes ( $p$  values ranging from 0.02-0.004). For chromosome 15, suggestive results were found for markers including and distal to D15S143, with maximum significance of 0.004 at D15S117 for single point analysis of a standardized articulation test. Suggestive multipoint results (GENEHUNTER2) were also found on chromosome 1 for a nonword repetition test, with a maximum NPL of 1.07 at D1S199. These results support the hypothesis that both dyslexia and SSD share common genetic mechanisms involving phonologic processing. Supported by NIH-NICHD 2 R01 MH38820 to BFP.

**Linkage and Association of Systemic Lupus Erythematosus (SLE) on 1q23.3.** *H. Wu<sup>1</sup>, R.M. Cantor<sup>2</sup>, J.M. Grossman<sup>1</sup>, A.A. Rumbin<sup>1</sup>, N. Shen<sup>3</sup>, C.S. Lau<sup>4</sup>, D.J. Wallace<sup>5</sup>, F.C. Arnett<sup>6</sup>, H.M. Badsha<sup>7</sup>, H.H. Chng<sup>7</sup>, B.H. Hahn<sup>1</sup>, B.P. Tsao<sup>1</sup>.* 1) Medicine,UCLA,LA,CA; 2) Human Genetics and Pediatrics,UCLA,LA,CA; 3) Renji Hosp.,Shanghai,China; 4) Queen Mary Hosp.,Hong Kong; 5) Cedars-Sinai Research Institute,LA,CA; 6) Univ. of Texas,Houston,TX; 7) Tan Tock Seng Hosp.,Singapore.

SLE is a complex, multifactorial autoimmune disease, and twin and family studies indicate that genetic factors play a key role in its pathogenesis. Evidence of SLE linkage to 1q22-24 has been reported in independent cohorts. We fine-mapped this region to provide additional support for linkage and localize the putative SLE susceptibility gene(s). Ten markers spanning 33cM of 1q22-25 were typed and analyzed by model-free, multipoint linkage analysis. Linkage to 1q23 peaked at D1S2675 (NPL 3.51,  $P = 0.0002$ ) in 115 multiethnic families with 143 affected sibpairs (ASP). When the sample was stratified by ethnicity, 76 non-Caucasian (NC) ASP linkage peaked at D1S2675 (170.8cM, NPL 3.41,  $P = 0.0003$ ), while in 67 Caucasian ASP linkage peaked at D1S1677 (175.6cM, NPL 2.44,  $P = 0.008$ ). Using a drop of one NPL score as a guide, we focused on 11cM of the linked region. Association was assessed for 11 markers in the region using the ETDT program. Overall of skewing allele transmission at D1S2844 (175.0cM,  $P = 0.0009$ ) and D1S1677 (175.6cM,  $P = 0.01$ ) was observed in 160 Caucasian trios, and at D1S484 (169.7cM,  $P = 0.008$ ) and D1S2878 (177.9cM,  $P = 0.02$ ) in 125 NC trios. Preferential transmission of FCGR2A-131R/H alleles was not observed when this sample augmented by an additional cohort of simplex families was stratified into 152 Caucasian and 133 NC simplex families. The two markers associated in NC are 8cM apart, suggesting the possibility of multiple susceptibility genes in that ethnic group. The region between the two markers associated in Caucasian contains novel positional candidate genes including RGS4 (regulator of G-protein signaling 4), RGS5 and CDCA1 (cell division cycle associated 1) that we are testing. FCGR2A-131R/H, an important candidate gene at 170.8cM was typed and tested for association. It appears not to be an SLE risk factor in this multiethnic cohort.

**Chromosome 7q linkage in autism: No evidence for strengthened signal by including parental information or stratification based on language delay.** *S.J. Spence, S.H. Kim, D.H. Geschwind, M. Alarcón, AGRE.* UCLA Psychiatry, Pediatrics, Neurology, LA, CA.

Results of whole genome scans of autism have implicated several regions on chromosome 7 as putative sites for autism susceptibility loci. Stratifying affected families by language endophenotypes and including parental language history information have strengthened original linkage signals in the 7q region (Bradford et al. 2001). QTL analysis of language delay in multiplex autism families also yielded evidence of linkage on 7q (Alarcón et al. 2002). To replicate and extend these findings we obtained parental language history on a subset of 150 families from the Autism Genetic Resource Exchange (AGRE). Proband diagnosis was based on the Autism Diagnostic Interview-Revised and includes both narrow and broad spectrum. Parental language delay was defined as history of language delay and/or problems learning to read. Genotypes including 48 microsatellite markers were available for the nuclear families. A multipoint nonparametric linkage analysis was performed using the diagnosis of autism spectrum disorder with the Genehunter program. Samples were analyzed in 3 ways: all families, language delayed families (i.e., two probands with word delay-age at first word > 18 months [WD] or phrase delay-age at first phrase > 36 months [PD]), and non-delayed families (i.e., less than two probands with word [NWD] or phrase [NPD] delay). These analyses were repeated including parental language information. The strongest linkage peak to autism for chromosome 7 in all families was at 147.55 cM (NPL=2.59), and a smaller peak was at 107 cM (NPL=1.45). Stratifying families as a function of proband language delay, either in word or phrase, did not strengthen the linkage signal. For example, the peak nonparametric linkage score for PD families (n=37; NPL=1.17, 154.39 cM) was lower than the peak score for NPD families (n=113; NPL=2.73, 147.55 cM). In addition, the NPL signal did not vary due to the presence of parent language information (Z=2.55, 147.55 cM). Therefore, parental language information or stratification based on speech delay did not significantly strengthen linkage to autism in this sample.

**Young-onset hypertension in Chinese is linked to STRP marker of lipoprotein lipase (LPL) and associated with its haplotype.** *W. Pan<sup>1</sup>, P. Chen<sup>1, 4</sup>, Y. Jou<sup>2</sup>, C.S.J. Fann<sup>1</sup>, J. Chen<sup>3</sup>, C. Chung<sup>1</sup>, Y. Chen<sup>1</sup>, S. Wu<sup>4</sup>, M. Kang<sup>1</sup>, C. Lin<sup>1</sup>.*  
1) Inst Biomedical Sci, Academia Sinica, Taipei, Taiwan; 2) National Health Research Inst, Taipei, Taiwan; 3) Veteran General Hospital, Taipei, Taiwan; 4) National Defense Medical College, Taipei, Taiwan.

Young-onset hypertension is associated with moderate obesity and borderline elevation of fasting triglyceride (TG) in Chinese. Therefore, LPL, a major metabolizing enzyme of TG, has been selected as one of 18 candidates in our efforts to map hypertension genes.

Young-onset hypertension was defined as essential hypertension developed prior to age 40. Using data from 59 families, the initial ASP analysis showed a positive linkage signal for D8S1145 near *LPL*. The second stage analysis with more STRP markers showed a significant linkage (NPL score=3) and TDT-association ( $p < 10^{-5}$ ) with LPL marker located in intron 6 of *LPL*. In order to identify variants of *LPL* in Taiwanese, we sequenced 5 flanking region and exons of *LPL* for 41 subjects. Two cSNPs, a silent mutation in exon 8 and Ser447X in exon 9, were verified. By sequencing another 12 individuals, 8 new iSNPs were discovered from intron 1 to intron 3. In addition, 38 iSNPs were verified and 5 new iSNPs discovered from the exon 4 to 3 flanking region. Two major LD blocks ( $D \geq 0.8$ ) were identified within 44 SNPs with minor allele frequency 0.07. Six haplotypes, each with 2-4 SNPs, were constructed using information on LD and their locations. A haplotype consisting of two cSNPs of exon 8 and 9 and one SNPs in the intron 7 was significantly lower in its transmission to the obese hypertension probands (no. of informative trios=39). We extended the analysis to another larger sample (no. of informative trios=151) obtaining consistent results of lower transmission to young-onset hypertension ( $p < 0.05$ ) and young-onset obese hypertension ( $p < 0.0003$ ). On the other hand, the haplotype consisting of the common SNP allele in exon 8 and the common allele of Ser447X significantly increased the risk of young-onset obese hypertension ( $p = 0.007$ ). Our results suggest that *LPL* may be involved in the pathogenesis of essential hypertension.

**Increased evidence for schizophrenia linkage to 8p after stratification by *DTNBPI* high-risk haplotype.** *B.T. Webb*<sup>1, 3</sup>, *M.C. Neale*<sup>1, 2, 3</sup>, *E.J.C.G. Van den Oord*<sup>2, 3</sup>, *A.H. Fanous*<sup>2, 3</sup>, *K.S. Kendler*<sup>1, 2, 3</sup>, *B.P. Riley*<sup>1, 2, 3</sup>. 1) Departments of Human Genetics and; 2) Psychiatry; 3) Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, VA, USA.

We have previously reported a schizophrenia linkage to 8p and more recently a highly significant association with SNPs and a high-risk haplotype in the *DTNBPI* gene on 6p using the Irish Study of High Density Schizophrenia Families (ISHDSF). The evidence for linkage on 8p was reexamined in light of the association evidence on 6p. When families containing the *DTNBPI* high-risk haplotype are removed, the evidence for linkage to 8p increases substantially and includes a maximum multipoint HLOD of 4.08 and a multipoint NPL Z-score (uncorrected) of 3.78. Conversely, the high-risk haplotype positive group has little evidence for linkage on 8p. The maximum difference in NPL Z-score between the *DTNBPI* high-risk haplotype positive and negative groups was 4.11. Monte Carlo stratification simulation showed that this pattern of results was unlikely to have occurred by chance (p-value <0.05). Null genome scan simulations were used to calculate the empirical genome-wide significance of the NPL results, which were significant (p-value 0.014, 95% C.I. 0.0077-0.02). This is the first report of genome-wide significant multipoint linkage results using the ISHDSF, currently one of the largest single study collections of schizophrenia pedigrees. These results provide further support for both a schizophrenia locus independent of *NRG1* on 8p and the *DTNBPI* high-risk haplotype association on 6p.

**Reevaluation and fine mapping of a Brazilian pedigree reveals another family with Allan-Herndon-Dudley syndrome.** *M.R. Passos-Bueno<sup>1</sup>, T.S. Zorick<sup>1</sup>, S. Kleimann<sup>2</sup>, M. Zatz<sup>1</sup>, S. Rosenberg<sup>2</sup>.* 1) CEGH, Dept Biol, Univ De Sao Paulo, Sao Paulo SP, Brazil; 2) Santa Casa Hospital, So Paulo, SP, Brazil.

Microsatellite-based fine genetic mapping of a previously published family affected by a severe form of X-linked mental retardation was performed (Passos-Bueno et al., 1993, *Am J Med Genet*, 46(2):172-175). Taking advantage of several informative recombinations, we were able to narrow down the critical region for the disease gene from 52 cM to an interval of approximately 13.4 cM (26.5MB) on the X chromosome, between the microsatellite markers DXS8080 (Xp11.2) and DXS986 (Xq13). Given that this genetic interval overlaps with that for Allan-Herndon-Dudley Syndrome (AHDS), which has been mapped to Xq12-Xq21, we performed a further neurological workup of affected members in this family. Neurological examination of patients from this family shows that from a clinical point of view, the pedigree under study is indistinguishable from AHDS. Based upon these results, we now propose that the original Brazilian pedigree actually represents another familial case of AHDS. This would restrict the candidate region for AHDS on the X chromosome to approximately 3 cM (10MB), containing at least 100 genes (NCBI). Eighteen genes mapped within this area (AR, CHIC1, CITED1, Dach2, EphrinB1, ARHGEF9, GPR23, HCA1, HCA3, ITM2A, KLHL4, NE-DLG3, NLG3, RagB, SNX12, TAF9L, ZNF261, ZNF6) were already tested by direct sequencing, however, no obvious mutations have yet been found. FAPESP, CNPq, CEPID.

Program Nr: 1955 from 2003 ASHG Annual Meeting

**Genotyping: Evaluation of inter-run sizing precision of the Applied Biosystems 3730 DNA Analyzer.** *B. Craig, M. Brehm, Y. Tsai, C. Boehm, E. Pugh, K. Doheny.* Center for Inherited Disease Research, IGM, JHUSOM, Baltimore, MD.

Microsatellite linkage studies of complex disease require the genotyping of thousands of samples. The capillary electrophoresis instrument being evaluated for use at the Center for Inherited Disease Research is the 3730 DNA Analyzer, which operates at higher efficiency and lower operating costs than the 3700 platform. The 3730 was developed with specifications of  $<0.15$  SD intra-run sizing precision, which allows for single base-pair resolution for sequencing applications. For microsatellite genotyping, inter-run variability is also a key factor because allelic sizing data is combined across multiple runs and instruments. Too much variability in inter-run sizing leads to the inability to bin data with confidence. Our experience with the 3700 platform is an inter-run variability of  $\sim 0.12$  SD using GS500-LIZ(-250) size standard and  $\sim 0.09$  SD using CIDR size standard. We first measured sizing precision of the 3730 instrument using GS500-LIZ(-250,-340) size standard, G5 installation standards, standard instrument running protocols, and unaltered lab environmental conditions over the period of a week (2 trials). The SD across 23 runs ( $n=1,104$ ) was as high as  $\sim 0.18$  SD. The variability across trial 1 was significantly higher than trial 2, with the most variation in the first 2 runs. Further tests identified key variables: temperature of the running buffer and laboratory. Preheating the 1X running buffer to 30 C immediately before starting runs significantly reduced the inter-run variability across 2 trials, to a maximum  $\sim 0.10$  SD. The additional impact of environmental temperature was tested by varying the laboratory temperature from 19 C to 23 C (while preheating 1X buffer to 30 C). Significantly lower inter-run variability were observed under the 23 C conditions ( $\sim 0.07$ SD). These findings suggest that optimal conditions of the 3730 for genotyping are pre-equilibrated 30 C running buffer in an environmental temperature of  $\sim 23$  C. Failure to control inter-run sizing for data combined across multiple runs will result in genotypes falling outside predefined allelic bins, significantly reducing the utility of automated allele calling software such as GeneMapper.

Program Nr: 1956 from 2003 ASHG Annual Meeting

**Multiplex PCR for microsatellite genotyping using an existing marker set.** *K. Hetrick, D. Hayden, B. Craig, B. Troup, J. Romm, C. Boyce, K. Doheny, C. Boehm.* Center for Inherited Disease Research (CIDR), IGM, Johns Hopkins Univ Sch of Medicine, Baltimore, MD.

CIDR uses 400 markers for a 10cM genome-wide scan. Until recently these were organized into 50 panels. With the availability of new dyes for Applied Biosystems (AB) platforms, we reorganized these markers into 38 panels (average 11 markers/panel), primarily focusing on dye color and product size. In this study, 373 markers (37 panels) were PCR'd both as a PCR reaction that combined all markers in the panel (multiplex or MP) and by themselves (singleplex or SP) using standard PCR conditions. Since there was a wide range of Tms within each panel (up to 11<sup>0</sup>C), we utilized a touch-down PCR to minimize non-specific products. Performance was evaluated on the AB 3730 DNA analyzer. Six categories were observed: Primer-pair (1) worked comparably in both reactions (217/373 = 58%); (2) had significant decrease in yield in the MP (23%); (3) worked well as a SP but failed in MP (1%); (4) worked poorly in SP, failed in MP (8%); (5) failed in both reactions (7%); and (6) unable to score (3%). Although product yield overall was lower in the MP, 24% of the markers (categories 2 and 3) showed significant decrease in MP. Despite expectation, there was not a good correlation between the G values and a primer-pairs performance. Other phenomena observed in the MPs included non-specific PCR products, a tendency for the product to fall out of the +A form (all R primers are tailed to favor the +A form), and an increase in allelic imbalance. Changing the extension temp from 72<sup>0</sup>C to 60<sup>0</sup>C eliminated many of the non-specific products. Lengthening the final extension to 90 minutes pushed products back into the +A form. We continue to investigate allelic imbalance. Primer reagent was shown to be stable at 4<sup>0</sup>C over at least 3 months. Of the 37 panels, 5 (14%) are utilized routinely now and 32 (86%) require modification of one or two primer-pairs. We believe these results indicate that high-level multiplexing will be achievable without having to redesign or replace most of our current markers.

**Refinement and Candidate Gene Screening of the Cerulean Cataract Type 1 Locus on 17q24-q25.** *M.B. Gorin<sup>1</sup>, B.W. Rigatti<sup>2</sup>, F.Y. Demirci<sup>2</sup>, S.R. Clarke<sup>2</sup>, T.S. Mah<sup>2</sup>, D.E. Weeks<sup>3</sup>, R.E. Ferrell<sup>3</sup>*. 1) Ophthalmology & Human Gen, Univ Pittsburgh, Pittsburgh, PA; 2) Ophthalmology, Univ Pittsburgh SOM; 3) Human Genetics, Univ Pittsburgh GSPH.

Cerulean cataracts (CCA) are autosomal dominant, juvenile-onset hereditary cataracts that are noteworthy for tiny blue or white opacities, predominantly in the lens cortex. There are three reported CCA loci: CCA type 1 on 17q24-q25, CCA type 2 on 22q11.2-q12.2, and CCA type 3 on 2q33-35. CCA2 and CCA3 were shown to be caused by a mutation in the -B2-crystallin gene and -D crystalline gene, respectively. The CCA1 locus was originally mapped in a single large family between microsatellite markers D17S802 and D17S836. Because of new data available since the original study, we have remapped the disorder in this family and tested several candidate genes. We used GenBank and other databases to establish a physical contig. We evaluated new microsatellite markers using ABI genotyping and SNPs using a combination of DHPLC (Transgenomic Wave), RFLP, and sequencing of the PCR products to detect alleles and refine the critical region. Potential candidate genes were evaluated for expression using the NEIBank lens cDNA database and by PCR in a human lens cDNA library and tested for mutations in exons and splice junctions by sequencing. We evaluated 7 known and 14 previously unreported microsatellite markers, as well as 16 previously reported SNPs and 6 novel SNPs within potential candidate genes spanning our critical region. In addition to the 7 known microsatellite markers, 7 of the 14 novel microsatellite markers and 16 of the 22 SNPs were informative in this family. Twelve possible candidate genes showed expression in a human lens cDNA library. Sequencing of the exons of these genes did not identify any disease-causing mutations. With the gradual completion of the Human Genome Project, this region of chromosome 17 is becoming better-defined. We have been able to further localize the CCA1 locus and establish that no known lens-specific genes are within the critical region. Elucidation of the causative gene for CCA1 has the potential of identifying a novel gene that contributes to lens biology.

**Determining phase of the poly-GT and poly-T repeat tracts in the CFTR gene by melting curve analysis of hybridization probes.** *A. Millson<sup>1</sup>, G. Pont-Kingdon<sup>1</sup>, S. Page<sup>1</sup>, E. Lyon<sup>1,2</sup>*. 1) ARUP Laboratories, Salt Lake City, UT; 2) Pathology Department, University of Utah.

Over 1000 mutations have been described in the CFTR gene. Although some of these mutations such as F508del are clearly classified as severe, the phenotype/genotype correlation of other mutations is not well established. Polymorphisms within the CFTR gene may modify the effects of some mutations. The poly-T tract located in the intronic region before exon 9 is one example. The number of Ts present, either 5,7, or 9, affects the splicing efficiency of exon 9. The combination of the R117H mutation in cis with the 5T polymorphism confers a severe CF allele, whereas R117H alone or in trans with the 5T may be mild. A tract of GT repeats immediately precedes the poly-T tract and may also influence the CF phenotype. We have developed a molecular assay to determine the chromosome phase (cis or trans) of the poly-T and poly-GT tracts at the 3' end of intron 8 (IVS-8) of the CFTR gene directly from an individual sample. The assay utilizes LightCycler melting curve analysis of a hybridization probe covering both the poly-GT and the poly-T region. The temperature at which the probe dissociates from the template is determined by the base composition between the target and the complementary probe. Distinct Tms were obtained for each combination of GT and T repeats. Bi-directional sequencing with BigDye terminator chemistry was used to confirm the polymorphisms and phase. In 90 samples screened, 7 distinct haplotypes were determined with 10, 11 and 12 GT repeats in varying combinations with the 5, 7 and 9 poly-T repeats. This assay demonstrates the ability of melting curve analysis to haplotype directly from a single individual.

**Evaluation of the Human Fas (-670 A/G) and Fas Ligand (-844 C/T) Single Nucleotide Polymorphisms in Scleroderma Patients from North America.** *Y. Zhang<sup>1</sup>, L. Wang<sup>1</sup>, B.S. Maher<sup>2</sup>, R.E. Ferrell<sup>3</sup>, P.A. Morel<sup>4</sup>, T.A. Medsger Jr.<sup>4</sup>.* 1) Dept Medicine, Div PACCM, Univ Pittsburgh Sch Med, Pittsburgh, PA; 2) Center Craniofacial Dental Genet, Univ Pittsburgh Sch Dent Med, Pittsburgh, PA; 3) Dept Human Genet. Univ Pittsburgh Grad Sch Public Health, Pittsburgh, PA; 4) Dept Medicine, Div Rheumatology, Univ Pittsburgh Sch Med, Pittsburgh, PA.

Scleroderma, or systemic sclerosis (SSc), is a group of heterogeneous disorders characterized by fibrosis of skin and internal organs. The etiology and pathogenesis of SSc is still largely unknown. The soluble Fas receptor (Fas) has been shown to be elevated in the sera of SSc patients in comparison with healthy controls, suggesting that impaired apoptosis may play roles in the development of SSc. To determine whether genetic polymorphisms in the Fas receptor and Fas ligand (FasL) genes are associated with SSc, we have evaluated the -670 A/G and -844 C/T SNPs in the promoter region of the Fas and FasL gene, respectively, in SSc patients from North America. The study cohort consists of 350 American Caucasians recruited from the University of Pittsburgh Medical Center with 224 of SSc patients and 126 of ethnicity-matched controls. The two SNPs were analyzed using TaqMan SNP method. Although it was not quite statistically significant, the frequency of AA genotype of the -670A/G SNP in the Fas gene was reduced in the SSc (23.2%) compared to normal controls (32.6%) with  $p = 0.0602$  in a Fishers exact test. Different genotype distributions of the -670 A/G SNP in the Fas gene were also detected between SSc (AA = 23.2%, AG = 54.1%, GG = 22.8%) and controls (AA=32.6%, AG=46%, GG=21.4%) with  $p = 0.156$  for the Chi-square analysis. In contrast, the allele frequency and genotype distribution for the FasL gene polymorphism (-844 C/T) are similar between the SSc and matched controls. Further characterizations of the -670 A/G polymorphism in additional SSc cohorts are essential to determine the significance of this polymorphism in the development of SSc.

**Association of Schizophrenia and a polymorphism in the 12-LOX gene.** *C. Fridman, E. P.B. Ojopi, S. P. Gregrio, H. Vallada, P. Saletti, J. Yacubian, W. Gattaz, E. Dias Neto.* Dept and Inst Psychiatry (IPq-FMUSP), Univ Sao Paulo, Brazil.

Schizophrenia (SC) is a psychiatry disorder affecting ~1% of the population. SC often develops in young adults previously normal, and is characterized by hallucinations, psychotic symptoms, inappropriate emotional responses, disordered thinking and concentration, social deterioration. The neurodevelopmental hypothesis to SC suggests that interaction between genetic and environmental events during early periods in neuronal growth may negatively influence the way by which nerve cells differentiated. Growing evidence indicates bipolar disorder (BP) and SC display considerable overlap in epidemiologic features. Family studies show that genes play an important role in the predisposition to SC, and reveal familial co-aggregation of the two disorders, suggesting a significant overlap in the genes contributing to them. SNPs are the most frequent variations found in the human genome, defined as punctual regions of DNA where nucleotides can vary among individuals, and are frequently used in studies of complex diseases. Our goal is to search non-synonymous SNPs in genes that can be associated with psychiatric diseases, and to investigate their frequencies in a series of patients. Due to the overlap among BP and SC, and to the association we recently found in BP patients for a SNP in 12-LOX gene, we test this same polymorphism to SC. The alteration is a G to A substitution that leads to a change of an Arginine to a Glutamine. We sequenced 134 SC patients and 148 control individuals, showing an increased frequency of heterozygous G/A among patients when compared to controls (35.1% in controls and 50% in cases;  $X^2 = 4.426$ ;  $P=0,035$ ) and a significant increase of allele A in individuals from this group (58.8% in controls and 70% in cases;  $X^2 = 3.952$ ;  $P=0,047$ ). This gene belongs to the lipoxygenase family, is involved in the leukotrienes pathway, is able to sensitise the nuclear membrane to be more susceptible to the release of arachidonic acid and is related to the apoptosis. Our results suggest an association of this polymorphism and SC in this Brazilian sample. Supported: FAPESP, ABADHS.

**Association of a Met/Thr Polymorphism in the Pigment Epithelium-Derived Factor Gene within a Population with Age-Related Macular Degeneration.** *B. Appukuttan<sup>1</sup>, T.J. McFarland<sup>1</sup>, B. Wilmot<sup>3</sup>, K. Rust<sup>1</sup>, Y. Zhang<sup>1</sup>, D.W. Schultz<sup>1</sup>, P. Francis<sup>1</sup>, P. Kramer<sup>2,3</sup>, M. Wirtz<sup>1</sup>, M.L. Klein<sup>1</sup>, J.T. Stout<sup>1</sup>.* 1) Casey Eye Institute; 2) Department of Neurology; 3) Department of Molecular & Medical Genetics, OHSU, Portland, OR.

Age-related macular degeneration (AMD) is common complex disorder in which progressive central retinal atrophy or hemorrhagic neovascularization result in visual loss. Pigment epithelium-derived factor (PEDF) is a retinal neurotrophic factor with antiangiogenic properties. It is thought that amino acid substitutions resulting from non-synonymous single nucleotide polymorphisms (nsSNPs) within the PEDF gene may alter the antiangiogenic potency of the protein, in turn predisposing to neovascular disease. The purpose of this study is to determine the frequency of such nsSNPs in patients with and without AMD. PCR products from 201 patients with AMD (neovascular AMD and geographic atrophy) and 172 age-matched controls were either sequenced or characterized by RFLP analysis. Nine SNPs are present within the protein coding sequence of the PEDF gene and of these, 4 result in amino acid changes. The nsSNPs are at codon 72 (Met/Thr), 132 (Pro/Arg) and 196 (Thr/Ile). All 373 individuals were homozygous for Thr-196. The nsSNP at codon 72 is highly polymorphic (49.3% heterozygous for Met/Thr-72), whereas very few individuals possess the homozygous allele coding for Arg-132. Depending on the presence of Met or Thr at codon 72 there is a gain/loss of a potential phosphorylation at Ser-93, which is situated within the putative receptor binding site. Chi-squared test was used in a test for homogeneity between AMD group and controls. AMD affected individuals were significantly more likely ( $p=0.44$ ) to carry at least one Thr-72 allele. Heterozygotes Met/Thr-72 were significantly more likely to be affected than homozygotes of either Met/Met-72 or Thr/Thr-72 ( $p=0.024$ ). Thus, there is a significant difference in the proportion of people that have the Met/Thr-72 genotype in this particular AMD population when compared to controls. Analyses are underway to characterize the precise functional implications of these changes.

**The ABCA1/G1051A polymorphism and Alzheimers disease in Whites and African Americans.** *P.P. Desai<sup>1</sup>, S.T. DeKosky<sup>1,2,3</sup>, H.C. Hendrie<sup>4</sup>, J.R. Murrell<sup>5</sup>, M.I. Kamboh<sup>1,2</sup>.* 1) Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Psychiatry, Univ Pittsburgh, Pittsburgh, PA; 3) Neurology, Univ Pittsburgh, Pittsburgh, PA; 4) Psychiatry, Indiana Univ, Indianapolis, IN; 5) Pathology and Laboratory Medicine, Indiana Univ, Indianapolis, IN.

A pathological hallmark seen in the brain of all Alzheimers disease (AD) cases is the deposition of -amyloid (A) plaques. Studies in transgenic animal models of AD show that elevated cholesterol levels increase A production, while treatment with cholesterol-lowering agents decrease A levels. Among the genetic loci implicated in late-onset sporadic AD, only the E4 allele of the apolipoprotein E (APOE) gene has been consistently shown to be associated with AD. APOE is also involved in brain cholesterol metabolism, further signifying the role of cholesterol in AD pathogenesis. Among other members of the cholesterol transport pathway, ATP-binding cassette transporter 1 (ABCA1) regulates the excretion of cholesterol from tissues. In the present study, we examined the role of the ABCA1/G1051A polymorphism in modifying the risk of sporadic late-onset AD in white and African American subjects. The cohorts were comprised of 695 white AD cases and 637 age-matched controls; and 128 African American AD cases and 213 age-matched controls. Among whites, frequency of the less common 1051\*A allele was significantly higher in female AD cases (n=470) compared to female controls (n=392), and the 1051\*A allele carriers showed a significant association with AD, with age and APOE-adjusted OR 1.63 (1.20-2.22; p=0.002). No significant difference in allele or genotype frequencies was seen in white male AD cases and controls. Frequency of the 1051\*A allele was significantly higher in African American AD cases than white AD cases (56.3% vs. 29.2%; p=0.0001), and in African American controls compared to white controls (58.5% vs. 27.6%; p=0.0001). There was no significant difference in allele or genotype frequencies in African American male or female AD cases and controls. In summary, we report a gender-specific association between the ABCA1/G1051A polymorphism and AD in whites however, no significant association was seen in African Americans.

**Interleukin 9 receptor gene polymorphisms in childhood wheezing and atopy.** *E. Melen*<sup>1,2</sup>, *H. Gullsten*<sup>2,3</sup>, *A. Lindstedt*<sup>2</sup>, *M. Zucchelli*<sup>4</sup>, *F. Nyberg*<sup>1,5</sup>, *G. Pershagen*<sup>1</sup>, *M. Wickman*<sup>6</sup>, *J. Kere*<sup>2,4</sup>. 1) Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; 2) Clinical Research Centre, Huddinge University Hospital, Stockholm, Sweden; 3) Department of Medical Genetics, University of Helsinki, Finland; 4) Centre for Biotechnology at Novum, Karolinska Institutet, Stockholm, Sweden; 5) AstraZeneca, Molndal, Sweden; 6) Department of Occupational and Environmental Health, Karolinska Hospital, Stockholm, Sweden.

Interleukin 9 (IL9) has many biological effects that are important for the development of allergic diseases. The IL9 receptor gene, located on the pseudoautosomal region of X and Y chromosomes, has previously been associated with asthma. Our aim was to study polymorphisms in the IL9 receptor gene and childhood allergic diseases. Infants born in Stockholm, Sweden (n=4,089), were recruited in a prospective study, BAMSE. Symptoms of allergic diseases were obtained at one, two and four years of age. Blood samples (n=2,614) were drawn at four years of age for analysis of specific IgE. Using a case-cohort design, 526 cases (wheezers) and 526 controls were selected for analyses of 26 IL9R single-nucleotide polymorphisms (SNPs). Twenty SNPs were non-polymorphic or had very low allele frequencies and 2 SNPs were excluded because they were not in Hardy-Weinberg equilibrium. The remaining 4 SNPs were found to be in strong linkage disequilibrium and within one haplotype block. Haplotype analyses with these SNPs showed significant associations with wheezing up to the age of four (most common haplotype 52.8% in controls vs 57.4% in wheezers, p=0.02 and second most common haplotype 18.9% vs 14.7%, respectively, p<0.01). The frequency of the second most common haplotype was also lower in children with specific IgE to airborne or food allergens (19.0% in non-sensitised vs 14.3% in sensitised children, p=0.02). Thus, both disease-associated and protective haplotypes were identified. Our data suggest that variations in the IL9R gene may influence susceptibility to childhood wheezing and atopy.

Program Nr: 1964 from 2003 ASHG Annual Meeting

**Family based and case-control association analysis of circadian gene polymorphisms in bipolar I disorder.** *H. Mansour, J. Wood, B. Devlin, T. Logue, K.V. Chowdari, D.J. Kupfer, V.L. Nimgaonkar.* Departments of Psychiatry and Human Genetics, University of Pittsburgh School of Medicine and Graduate School of Public Health, Western Psychiatric Institute and Clinic, Pittsburgh, Pennsylvania, USA.

Disruption in circadian rhythms is a hallmark of bipolar I disorder (BD1). Hence, we evaluated associations of BD1 with polymorphisms of genes mediating circadian function. We investigated 135 probands with BD1 (DSM IV criteria), as well as available parents. Cord blood samples from local live births served as unrelated, unscreened controls (n =182). The following genes are being investigated: Clock, Bmal1, Cry1, Cry 2, Per 1, Per 2, Per 3, Timeless, and CSNK1E. We have identified 45 informative SNPs from public databases and through our re-sequencing efforts. Assays for these polymorphisms have been developed and are based on multiplex PCR followed by single base extension analysis (SnaPshot assays, ABI). Out of eight SNPs genotyped at Bmal1, two showed significant transmission distortion. Significant transmission distortion of haplotypes bearing these SNPs was also present. Case-control differences reinforced these findings. Bmal1 is known to interact at the molecular level with Clock and Per 1. Remarkably, SNPs and haplotypes at these genes also displayed transmission distortion. Only one of the other six circadian genes showed any transmission distortion (Timeless). We are currently analyzing gene-gene interactions. These results suggest an etiological role for circadian genes in BD1.

**Comparison of SNP genotyping between RFLP analysis and LNA dual labelled probes for allele discrimination.**

*M.P. Johnson, L.M. Haupt, L.R. Griffiths.* Genomics Research Centre, Griffith University, Gold Coast, Queensland, Australia.

The analysis of single nucleotide polymorphisms (SNPs) can be achieved via a vast array of methodologies. More rapid and cost effective assays are sought that are as reliable as conventional genotyping efforts such as RFLP analysis. This study compared RFLP analysis and locked nucleic acid (LNA) dual labelled probes to confirm DNA pooling results obtained for several SNPs in a migraine population-based association cohort. Selected SNPs underwent initial screening using DNA pooling and the ABI Prism SNaPshot Multiplex Reaction Kit. Allele frequency estimates obtained from the DNA pools were then confirmed by individual genotyping. RFLP analysis identified a *Sau96I* restriction site for an AG SNP. Genotyping for this SNP underwent conventional PCR and restriction digest protocols prior to agarose gel electrophoresis. With no naturally occurring restriction enzyme recognition sequence for a second SNP (CT) screened by DNA pooling, a dual labelled LNA probe set was designed (Proligo LLC). SNP allelic discrimination using the LNA probe set was optimised using real-time PCR analysis and confirmed by ABI BigDye Terminator Sequencing. RFLP analysis for the AG SNP confirmed individual allele frequencies (A 57.2%; G 42.8%) to those of the estimated pooled allele frequencies (A 58.7%; G 41.3%) for the migraine group (n = 358). Similarly, individual allele frequencies (A 54.5%; G 45.5%) and estimated pooled allele frequencies (A 52.4%; G 47.6%) were replicated for the control group (n = 372) using RFLP analysis. Sequencing confirmed the SNP allelic discrimination using the LNA probe set and real-time PCR analysis for the CT SNP (n = 9, 4 CC homozygotes, 5 CT heterozygotes). Initial analysis using a dual labelled LNA probe set has proven effective in SNP genotype calling. In particular, the use of LNA probes is appropriate for target sequences containing no naturally occurring restriction enzyme recognition sequence. Considerably less time and effort to genotype individual DNA samples is involved when using LNA probe/real-time PCR analysis, with increased reliability of SNP genotype calling evident for the LNA probe/real-time PCR assay.

Program Nr: 1966 from 2003 ASHG Annual Meeting

**An association of the FBN1 gene polymorphism with tall stature.** *M. Mamada, T. Yorifuji, K. Kurokawa, M. Kawai, T. Nakahata.* Dept Pediatrics, Kyoto Univ Hosp, Kyoto, Japan.

Contribution of genetic factors for controlling stature is estimated around 80%, however the major genes have not been fully determined. In our study to identify genes controlling stature, we carried out an association study targeting subjects with tall stature. We have analyzed 3 single nucleotide polymorphisms (SNPs) in each 6 candidate genes; FBN1, GH1, GHR, SHOX, FGFR3 and LH. SNPs were genotyped in healthy Japanese men grouped into Tall Stature (TS, N=150) and Normal Stature (NS, N=150). Tall stature was defined by final height over 180 cm (over 2SD), and normal stature by final height between 165 and 175 cm (-1 SD to 1 SD). Chi-square test with Bonferroni correction was used for statistical analysis. Haplotypes were reconstructed by means of a Bayesian algorithm implemented in the program Haplotyper in order to confirm that the selected SNPs were appropriate for the analysis. We demonstrated a significant association between SNP2 (T2008C) in FBN1 and tall stature ( $p=0.012$ ). In TS group, men with CC allele were significantly taller than those with TT. On the other hand in NS group, no significant differences were found. SNPs in other 5 genes were not statistically significant. An association between FBN1 gene polymorphism and stature has not been reported before. We cannot describe the exact mechanism by which this silent mutation affect stature. To clarify this point further investigation will be needed. In conclusion, our results suggest that variation of FBN1 may be one of the important determinant of stature.

**FEB2 positional candidate screening: ion channel associated genes on c19p.** *L. Nowak*<sup>1</sup>, *T. Stoffer*<sup>1</sup>, *D. Stone*<sup>2</sup>, *E. Johnson*<sup>1</sup>. 1) Neurogenetics Research, Barrow Neurological Institute, Phoenix, AZ; 2) CuraGen Corporation, Branford, CT.

Febrile seizures are the most common form of seizure disorder. 2-5% of all children will experience a febrile convulsion (FC) before the age of 5 (up to 15% in certain Asian populations), and as many as 7% of these children will go on to develop nonfebrile seizures and epilepsy later in life. This is 2-10 times the rate in the general population and a potentially serious health risk factor.

Loci for several potential febrile convulsion genes have been documented: FEB1 on chromosome 8q in a single Australian family; FEB2 on chromosome 19p in several large families from the Midwest, the East Coast and China; FEB3 (GEFS+1) which is associated with a mutation in the sodium channel subunit, SCN1B; FEB4 (GEFS+2) identified in April of 2000 as SCN1A, the sodium channel, neuronal type I, alpha subunit; FEB5 on chromosome 5q in a number of Japanese families; and finally FEB 6, localized to 6q22-24 in a European family.

The refinement of the C19p physical map, which includes the FEB2 critical interval, is essentially completed. A number of ion channel candidate genes map to this region of 19p and are undergoing mutational analysis as potential FEB2 candidates. One positional candidate gene, coding for a protein associated with the NMDA receptor shows a particularly high level of polymorphism. The results from the screening of this positional candidate will be presented.

**Detection and characterization of human *TPH2* variants.** Z. Zhou, R. Lipsky, D. Goldman. Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Rockville, MD.

Serotonin is a neurotransmitter that is involved in a wide variety of central nervous system (CNS) functions, including sleep, mood, pain, food intake, alcohol and drug abuse, and sexual behavior. Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the biosynthesis of serotonin. TPH2 was recently identified and shown to be responsible for serotonin synthesis in brain. We screened *TPH2* for sequence variants in 95 ethnically and clinically diverse individuals using denaturing high performance liquid chromatography (DHPLC) and direct sequencing, and examined the frequencies of some of these variants across several populations. Nineteen PCR amplicons were produced, covering 4.25 kb of the promoter, coding exons and their flanking intronic regions, and 3-untranslated sequences. PCR products were screened for single nucleotide sequence polymorphisms (SNPs) by detecting heteroduplex DNAs from heterozygotes. Amplicons revealing heteroduplexes were then directly sequenced. Sixteen SNPs were detected in the 4.25 kb region. Among the 16 SNPs, three were previously reported by NCBI dbSNP and Celera. Thirteen SNPs discovered in our study were new, four of which are located in the promoter, four in the exons, two in the introns, and three in 3-untranslated region. Heterozygosities for each of the SNPs were also calculated. Six SNPs with relatively high heterozygosities were selected for large scale genotyping using a 5nuclease assay. A total of 722 individuals from three ethnic groups, consisting of 198 African American, 180 Finnish Caucasians, and 344 U.S. Caucasians, were genotyped. Four of the six SNPs showed significant differences in allele frequencies among different ethnic groups, especially between the Finns and African Americans. We are now genotyping additional SNP markers spanning the 93 kb region of *TPH2* for haplotype and linkage disequilibrium analyses.

**A data mining tool for screening and analyzing single nucleotide polymorphisms (SNPs) in the human genome.** Z. Zhao<sup>1,2</sup>. 1) Human Genetics Center, University of Texas at Houston Health Science Center, Houston, TX 77030; 2) Keck Center for Computational and Structural Biology, Houston, TX 77030.

Single nucleotide polymorphisms (SNPs) are main genetic variations in the human genome and are valuable tools for identifying disease-associated genes, understanding the molecular mechanisms of mutation, and revealing the evolutionary history of human populations. With more than four millions of single nucleotide polymorphisms (SNPs) identified in the human genome, it is difficult to manually select and analyze SNPs from a large region or from various genes. Currently, the researcher is largely restricted to perform the basic search function in the public or private SNP databases. To data mine SNPs efficiently, a SNP database was designed and implemented by MySQL and a web interface was developed to allow the user to retrieve, search, and analyze the data from millions of SNPs. Besides the basic search of SNPs or genes, the user can screen a list of genes having the highest number of SNPs or the highest SNP density in a selected genomic region, a chromosome, or the whole genome. Other utilities include the statistical analyses of the substitutions, the ratio of transition over transversion, and gene structure categories of the SNPs in a restricted region or the genome. Furthermore, using the functional criteria of the genic categories, the user can obtain a fixed (say, 5) number of functional candidate SNPs for each gene within a large genomic region, which is critical in such projects as large-scale genotyping the complex disease genes. Such utility has been applied to screen approximately 10,000 functional candidate SNPs from all the SNPs available on chromosome 19. Finally, the basic or advanced queries can be performed directly on the database by users special requirements. This application can be easily extended to other functionalities for the genetic study if more SNP-related data are included. In conclusion, it provides a useful and efficient data mining tool for the researchers who study SNP patterns or search candidate SNPs in disease-causing genes.

**Polymorphisms in the genes of interleukin 10 and its receptors in association with inflammatory bowel diseases.**

*L. Zhang*<sup>1</sup>, *D.P. McGovern*<sup>1, 2</sup>, *T. Ahmad*<sup>1</sup>, *D.P. Jewell*<sup>1</sup>. 1) Gastroenterology Unit, NDM, Oxford University, Oxford, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, United Kingdom.

Interleukin 10 (IL10) is a down-regulatory cytokine that has been used in the treatment of Crohns disease (CD) and ulcerative colitis (UC). The overall response in clinical trials has been disappointing, but the clinical response might be confounded by the influence of genetic variation in the IL10 promoter or in its receptors. Analyses of polymorphisms in the IL10 gene and its receptors, IL10RA and IL10RB, might allow targeting of therapy to those patients who will respond. Furthermore, the IL10 knockout mouse is highly susceptible to the development of colitis. For these reasons the genes encoding IL10 and its receptors are attractive candidate genes for IBD. In this study, a total of 26 SNP markers have been examined: 9 located in the IL10 gene, 5 in IL10RA and 12 in the cytokine receptor cluster on chromosome 21q22.11. This cluster contains four genes that encode for IL10 RB and the subunits of the receptors for interferon-alpha, -beta and -gamma genes. Genotyping was performed with Sequenome in 376 UK Caucasians with CD, 380 with UC and 384 health controls. A strong association was seen between CD and IL10/+1812-A/A genotype ( $P = 0.0009$ , OR 6.1, 95%CI 1.8-20.8). Subanalysis showed no association with extent of disease, but this genotype was seen more commonly in those without peri-anal disease compared to patients with peri-anal disease ( $P = 0.014$ , OR 3.8, 95%CI 1.26-11.47). The wild type +1812-C/C appeared to be protective for UC ( $P = 0.0006$ , OR 0.566, 95%CI 0.4108-0.7785), but UC was associated with a coding polymorphism IL10RA/A60A-G/G ( $P = 0.005$ , OR 1.74 CI 1.184-2.56). There were very weak associations between polymorphisms of IL10 (-1387), IFNAR2 (-15843) and IFNGR2(+33543) and CD ( $P = 0.026, 0.035, 0.036$ , respectively), but no other associations were seen. These findings provide support for the role of IL10 in the pathogenesis of CD and UC. The biological significance of these mutations needs to be investigated.

Program Nr: 1971 from 2003 ASHG Annual Meeting

**Association of IL-13 -C1055T polymorphism with Asthma and Atopy in African-American patients.** *B. Chinoy, J. Moissidis, K. Yanamandra, D. Napper, T.F. Thurmon, J.A. Bocchini Jr., S. Bahna.* Dept. Pediatrics, LSU Health Sciences Center, Shreveport, LA.

Asthma and atopy are multifactorial diseases with genetic and environmental factors involved in their etiology. Several chromosomal loci have been implicated in the etiology such as chromosome 5q31-33, 12q15-24, and 11q13. Chromosomal region 5q31-33, contains several cytokine genes including IL-4, IL-5, IL-13, etc. Earlier findings have suggested a strong genetic influence of the chromosome 5q31 region in the development of allergic asthma. Immunoglobulin E synthesis is dependent on IL-4 and to lesser extent on IL-13. IL-5 induces eosinophilia. Because of their biological effects, IL-4 and IL-13, located at close proximity to 5q31, are very likely candidates in the inheritance of allergic asthma. For the first time, van der Pouw Kraan and his associates in 1999 have identified a single nucleotide polymorphism (SNP) in the promoter region of IL-13 gene, -C1055T. They have associated this SNP with increased risk of allergic asthma in the Dutch Caucasians. In the year 2000, van der Pouw Kraan et al have associated -C1055T polymorphism also with chronic obstructive pulmonary disease (COPD). Although several studies were conducted in Caucasians, there was limited literature on the association of this polymorphism with asthma or atopy in Africans or African-Americans. In the present investigation, we began the SNP studies on interleukins in the search for genetic markers in asthma and atopy patients in our local Northwest Louisiana African-Americans. We evaluated IL-13 -C1055T SNP genotyping in over 30 patients and compared the genotypes from 100 control infants. Our pilot data showed 24% -1055TT homozygotes in our patients compared to 12% in controls ( $p < 0.03$ , odds ratio 4.8). Mutant alleles also were significantly higher in patients than in controls 54.6% vs 37.9% ( $p < 0.03$ , odds ratio 2). Based on our data we believe that the 1055T mutant genotypes are a risk factor in the etiology of asthma and atopy in African-Americans. This is the first report on IL-13 C1055T in this community. Detailed data will be presented.

**Linkage and Association Analysis of COPD-related Phenotypes on Chromosome 19.** *J.C. Celedon*<sup>1,2</sup>, *B. Raby*<sup>1,2</sup>, *L. Palmer*<sup>1,2</sup>, *J. Sylvia*<sup>1</sup>, *D. Kwiatkowski*<sup>1,2</sup>, *H. Chapman*<sup>3</sup>, *M. Hernandez*<sup>1</sup>, *F. Speizer*<sup>1,2</sup>, *S. Weiss*<sup>1,2</sup>, *E.K. Silverman*<sup>1,2</sup>. 1) Channing Lab, Brigham & Women's Hosp, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) University of California at San Francisco, San Francisco, CA.

To study genetic determinants of chronic obstructive pulmonary disease (COPD), we enrolled 72 pedigrees ascertained through severe, early-onset COPD probands (FEV1 < 40% predicted [pred], age <53 yrs, without severe AAT deficiency). Results of a previously reported genome-wide linkage analysis for COPD-related traits in this population suggested that post-bronchodilator (post-BD) spirometric measures were optimal COPD-related phenotypes (Hum Mol Genet 2003;12:1199-1210). Following this genome scan, we conducted linkage analysis of 15 STR markers on chromosome (chr.) 19 (range, 33 to 68 cM) with post-BD quantitative (FEV1 and FEV1/FVC) and post-BD qualitative (mild airflow obstruction [FEV1 <80% pred and FEV1/FVC <90% pred], and moderate airflow obstruction [FEV1 <60% pred and FEV1/FVC <90% pred]) COPD-related phenotypes in 585 members of the COPD pedigrees. We used SOLAR for variance component linkage analysis of quantitative traits and the NPL all statistic (in MERLIN) for the analysis of qualitative traits. The maximum multipoint LOD scores were: 1.91 (at 63 cM) for FEV1; 1.87 (at 62 cM) for FEV1/FVC; 1.49 (at 42.3 cM) for mild airflow obstruction; and 2.41 (at 43.3 cM) for moderate airflow obstruction. To follow up on these results, we performed a family-based association analysis between four SNPs in a candidate gene in the linkage peak on chr. 19 (TGF1) and COPD-related traits using FBAT. Preliminary analyses have shown no significant association between any of the SNPs tested and qualitative COPD-related traits. Thus, we have found suggestive evidence for a COPD susceptibility locus on chr. 19, but our preliminary analyses do not support an association between TGF1 and COPD-related phenotypes. This abstract is funded by NIH Grants: HL61575 (EKS) and HL04370 (JCC).

**A first locus for isolated autosomal recessive optic atrophy (ROA1) maps to chromosome 8q.** *F. Barbet<sup>1</sup>, S. Gerber<sup>1</sup>, S. Hakiki<sup>1</sup>, I. Perrault<sup>1</sup>, S. Hanein<sup>1</sup>, D. Ducroq<sup>1</sup>, G. Tanguy<sup>1</sup>, J.-L. Dufier<sup>2</sup>, A. Munnich<sup>1</sup>, J.-M. Rozet<sup>1</sup>, J. Kaplan<sup>1</sup>.* 1) Unit de Recherches sur les Handicaps Gntiques de l'Enfant,INSERM U393, Hpital des Enfants Malades, Paris, France; 2) Service d'Ophtalmologie, Hpital des Enfants Malades, Paris, France.

In contrast to the frequent dominant optic atrophies where the neuropathy is usually an isolated event, isolated recessive optic atrophies (ROA) are very uncommon and have been described as severe congenital or early infantile conditions. To date, two loci for isolated dominant optic atrophy have been mapped, of which one was ascribed to mutations in the dynamin-like gene (OPA1). Conversely, no isolated autosomal recessive optic atrophy locus has previously been localised. Here, we report a large multiplex consanguineous family of French origin affected with an early onset but slowly progressive form of isolated optic atrophy. A genome-wide search for homozygosity allowed the mapping of the disease-causing gene to chromosome 8q21-q22 (Zmax of 3.41 at theta=0 for D8S270), in a 12 Mb interval flanked by markers D8S1702 and D8S1794. This localisation excludes allelism of the disease with isolated dominant optic atrophies and all known syndromic forms of recessive optic atrophy. This study supports the mapping of a first gene for isolated autosomal recessive optic atrophy (ROA1) to the long arm of chromosome 8.

**Expanding the genetic heterogeneity of keratoconus and posterior polymorphous dystrophy.** *N. Noordeh*<sup>1</sup>, *C.E. Willoughby*<sup>1,2,3,4</sup>, *V.E. Di Giovanni*<sup>1</sup>, *G. Billingsley*<sup>1</sup>, *S.B. Kaye*<sup>4</sup>, *E. Héon*<sup>1,2</sup>. 1) Genetics and Genomic Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) Dept of Ophthalmology and Vision Sciences, Hospital for Sick Children, Toronto, ON, Canada; 3) Dept of Medicine, University of Liverpool, Liverpool, UK; 4) Royal Liverpool University Hospital, Liverpool, UK.

Keratoconus (MIM#148300) is the most common cause of corneal transplantation in the Western world. We previously identified mutations in the human visual system homeobox (*VSX1*;MIM#605020) gene in keratoconus and also posterior polymorphous dystrophy. *VSX1* is the only keratoconus gene identified to date, but a keratoconus locus has recently been reported on chr16q 22.3-q23.1. Posterior polymorphous dystrophy (PPD;MIM#122000) is a progressive disorder of the corneal endothelium which is normally dominantly inherited and may co-exist with keratoconus. We reported mutations in *VSX1* in patients with both PPD and keratoconus, and mutations in *COL8A2* (MIM#120252) have been described in PPD. Eleven family members (5 affected and 6 unaffected) of a three generation Caucasian family from the UK with PPD and keratoconus were recruited. All individuals had a full ocular assessment including corneal topography and pachymetry. The diagnosis in the proband was histologically proven by light microscopy and immuno-histochemistry of corneal material obtained following corneal transplantation. This family was excluded from the *VSX1* and *COL8A2* loci by haplotype and linkage analysis. Microsatellite markers for the 16q22 loci, a recently documented keratoconus locus, were selected to cover the 6cM region on chr16q22.3-q23.1 (markers D16S2624, D16S3059, D16S512, D16S3018, D16S515 and D16S3138) for genotyping. Haplotype segregation has also excluded this family from the 16q22 locus. The genetic analysis of this family further expands the genetic heterogeneity of keratoconus and PPD.

**Optineurin sequence variants do not contribute to high-tension primary open-angle glaucoma.** *J.W. Walter<sup>1</sup>, R.R. Allingham<sup>1</sup>, J.D. Flor<sup>1</sup>, K.R. Abramson<sup>1</sup>, F.L. Graham<sup>1</sup>, C.S. Cohen<sup>1</sup>, C. Santiago<sup>1</sup>, E.A. del Bono<sup>2</sup>, J. Auguste<sup>2</sup>, K. Rogers<sup>2</sup>, J.L. Haines<sup>3</sup>, M.A. Pericak-Vance<sup>1</sup>, J.L. Wiggs<sup>2</sup>, M.A. Hauser<sup>1</sup>.* 1) Duke University, Durham NC; 2) Massachusetts Eye and Ear Infirmary, Boston MA; 3) Vanderbilt University, Nashville TN.

Recent studies have suggested that sequence variants in the optineurin gene (OPTN) increase susceptibility to normal-tension glaucoma (NTG), in which patients exhibit optic nerve damage without elevated intraocular pressure (Rezaie et al., *Science* 295: 1077). Two OPTN variants have been observed in multiple probands: the disease allele E50K, and the risk-associated allele M98K. To confirm the role of the E50K variant in NTG, we screened a panel of 50 NTG patients for this allele. E50K was observed in a single NTG patient, consistent with the interpretation of E50K as a NTG disease allele. To determine whether OPTN variants contribute to the more common high-tension primary open-angle glaucoma (POAG), we performed mutation analysis on our collection of 86 POAG families. Exons 4 and 5, containing these two variants, were sequenced using the probands from these families. E50K was observed in 0 of 86 high-tension POAG probands, suggesting that this allele does not contribute to glaucoma in this clinically distinct population. M98K was observed at the same frequency in POAG, NTG, and control populations, suggesting that this allele does not confer any additional risk of glaucoma. The remaining OPTN coding exons were screened for variations using denaturing HPLC analysis. No additional coding variants were detected in our sample set. A number of intronic SNPs were detected, none of which are anticipated to cause splice defects. These findings indicate that NTG and POAG do not have significant genetic overlap, and should be considered genetically discrete disorders. Our evidence indicates that OPTN variations do not contribute to high-tension POAG. To determine the effect of ethnic background on the frequencies of OPTN polymorphisms, and their possible roles in POAG, we have collected an additional 148 POAG patients from Ghana, the ethnic background of whom is similar to African American patients enrolled in our study.

**Candidate gene polymorphisms analysis and acquired skewing of X-chromosome inactivation in an elderly female population.** *P. Chagnon, S. Provost, M. Lussier, V. Bolduc, L. Busque.* HemaX Genome, Hopital Maisonneuve-Rosemont, Montreal, Quebec, Canada.

To equalize X-linked gene dosage between XX females and XY males, mammals have developed a unique dosage compensation mechanism that inactivates one of the two X-chromosomes in every female somatic cell. Females are in theory mosaics with an equal proportion of cells with the paternal X-chromosome in the active state and the other half with the maternal X chromosome in the active state. Significant deviation from the theoretical 1:1 ratio between paternal and maternal X chromosome is rare. However, Busque et al. (1996) have discovered that blood cells present an increased incidence of skewing of X-inactivation ratio over time. In fact, ratios greater than 3 to 1, occurs in 38 to 56% of normal females over 60 years of age. The exact aetiology of this age-associated skewing of blood cells is still unknown. Several recent observations point to an X-linked genetic basis to this phenomenon. We believe that gene(s) responsible for this phenotype are probably cell growth factors that favor cells harbouring either the activated maternal or paternal X chromosome. In order to test this hypothesis, we have analyzed, by a SNPs genotyping approach, 6 different genes located on the X chromosome that could be implicated in the phenomenon of X chromosome inactivation skewing. GATA1 (globin transcription factor 1), BMX (bone marrow kinase, X-linked), CYBB (cytochrome b-245, beta polypeptide), WAS (Wiskott-Aldrich), PIGA (phosphatidylinositol glycan, class A) and BM042 (uncharacterized bone marrow protein). We have recruited for this study 850 independent women from French Canadian population. X inactivation ratio was determined by the HUMARA test. Using TaqMan technology (ABI 7000), we have genotyped 48 different SNPs in the 380 most informative women (i.e. women who are highly skewed and normal women). Statistical analysis was performed by a chi-square based association test and also by the Fishers Exact Test using genotype frequencies. The results presented here show that the 6 genes investigated are not the cause of the age-associated skewing of blood cells.

**RHD maternal-fetal genotype incompatibility and schizophrenia: extending the MFG test to include multiple siblings and birth order.** *P. Kraft*<sup>1</sup>, *C.G.S. Palmer*<sup>2,3</sup>, *J.A. Woodward*<sup>3,4</sup>, *J.A. Turunen*<sup>5,7</sup>, *S. Minassian*<sup>1</sup>, *T. Paunio*<sup>7</sup>, *J. Lönnqvist*<sup>8</sup>, *L. Peltonen*<sup>5,7</sup>, *J. Sinsheimer*<sup>1,5,6</sup>. 1) Dept Biostatistics, Univ California, Los Angeles, Los Angeles, CA; 2) Dept Psychiatry and Behavioral Sciences, Univ California, Los Angeles, Los Angeles, CA; 3) Dept Statistics, Univ California, Los Angeles, Los Angeles, CA; 4) Dept Psychology, Univ California, Los Angeles, Los Angeles, CA; 5) Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA; 6) Dept Biomathematics, Univ California, Los Angeles, Los Angeles, CA; 7) Dept Molecular Medicine, National Public Health Institute, Helsinki, Finland; 8) Dept Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland.

Rh incompatibility disease has been implicated as a risk factor for schizophrenia. Here we extend the maternal-fetal genotype incompatibility (MFG) test used in a previous case-parent trio study of RHD maternal-fetal genotype incompatibility in a large Finnish schizophrenia sample. We modify the MFG test to include any number of siblings. The additional subjects not only give us greater power, they also enable us to model the impact of previous RHD maternal-fetal genotype incompatible pregnancies on the relative risk of genotype incompatibility in later-born siblings. This modeling is important, because RHD maternal-fetal genotype incompatibility is a proxy for Rh incompatibility disease, and the risk of Rh incompatibility disease increases with the number of previous genotype-incompatible pregnancies. We apply the modified test to available data from the Finnish sample. The best-fitting models are consistent with the hypothesized effect that previous incompatible pregnancies increase risk of schizophrenia due to RHD maternal-fetal genotype incompatibility. There was significant evidence that the relative risk of schizophrenia in the second- and later-born RHD maternal-fetal genotype incompatible children is 1.7, consistent with earlier estimates. Our extension of the MFG test has general application to family-based studies of maternal-genotype and maternal-fetal genotype interaction effects.

**Inheritance of expression profiles in MS families: A family based microarray analysis reveals higher similarity in the expression profiles of related individuals for the genes of the linked region.** *J. Saarela<sup>1</sup>, J. Hollmen<sup>2</sup>, D. Chen<sup>3</sup>, P. Tainola<sup>1</sup>, A. Jokiahho<sup>3</sup>, A. Palotie<sup>3,4</sup>, H. Mannila<sup>5</sup>, L. Peltonen<sup>1,3</sup>.* 1) Dept Molecular Medicine, Natl Public Health Inst, Helsinki, Finland; 2) Lab of Computer and Information Science, Helsinki University of Technology, Finland; 3) Dept. of Human Genetics, UCLA, CA; 4) Finnish Genome Ctr, Univ of Helsinki, Finland; 5) Helsinki Inst. of Information Technology, Finland.

We performed a genome-wide profiling of gene expression in four pedigrees to monitor whether a familial correlation existed in expression profiles. Analyzed families represented multiplex MS families showing linkage to a locus on 17q22-q24. Peripheral blood lymphocytes of 20 individuals were fractionated to CD4 positive and CD4 negative cells. Total RNA was extracted from cell populations and analyzed using the Affymetrix U133A microarrays. For comparison of expression profiles, we used the linear correlation coefficient between the profiles as a measure of similarity. As an aggregate quantity of similarity for families, we took the average of pairwise correlations between the expression profiles. In addition, we calculated the expected frequency of shared alleles between family members from the pedigree structure and used this as additional information when weighting the correlations. The profiles of gene sets (all genes, 25 mostly expressed genes, 500 randomly selected genes, genes with highest standard deviation, HLA genes mapping to 6p, genes mapping to 17q22-q24) were used in the comparisons. To test our hypothesis of average correlation in expression being stronger for families than for any other sets, we performed a permutation test. We consistently observed higher similarity between relatives than non-relatives in the expression profiles of 17q genes, while the other sampling groups of genes showed more variable findings. According to the permutation test (estimated with 100000 randomized samples), the familial correlation was statistically significant, both between family members ( $p=0.048$ ) and when the correlations were weighted using the pedigree structure information ( $p=0.015$ ).

**Maternally inherited aminoglycoside-induced and non-syndromic deafness associated with the novel C1494T mutation in the mitochondrial 12S rRNA gene in a large Chinese family.** *M.X. Guan*<sup>1,2</sup>, *H. Zhao*<sup>1,2</sup>, *R. Li*<sup>1</sup>, *Q. Wang*<sup>2</sup>, *Q. Yan*<sup>1</sup>, *W.Y. Yang*<sup>2</sup>. 1) Div Human Genetics, Children's Hosp Medical Ctr, Cincinnati, OH; 2) Department of Otolaryngology, Head and Neck Surgery, Chinese PLA General Hospital, Beijing 100853, China.

Mutations in the mitochondrial 12S rRNA gene have been shown to account for the significant cases of aminoglycoside ototoxicity. We report here the clinical, molecular and biochemical characterization of a large Chinese family with maternally transmitted aminoglycoside induced and non-syndromic deafness. Maternal members of this family showed the variable severity and age-of-onset in hearing impairment. Sequence analysis of the complete mitochondrial genome in this pedigree identified a homoplasmic C-to-T transition at position 1494 (C1494T) in the 12S rRNA gene. The C1494T mutation is expected to form a novel 1494U-A1555 base-pair, which is the same position as the 1494C-G1555 pair created by deafness-linked A1555G mutation, at the highly conserved A-site of 12S rRNA. This site has been implicated for the main target of aminoglycoside toxicity. Thus, it is anticipated that this alteration in the tertiary structure of 12S rRNA may lead to the sensitivity to aminoglycosides. Here, the sensitivity to aminoglycosides paromomycin or neomycin has been analyzed in lymphoblastoid cell lines derived from four deaf individuals and two hearing individuals from this Chinese family carrying C1494T mutation and four unrelated controls. In the presence of high concentration of paromomycin or neomycin, the C1494T mutation-carrying cell lines derived from Chinese family exhibited a significant average increase in doubling time, when compared to control cell lines. Furthermore, this mutation was absent in 300 unrelated controls, suggesting specific segregation with the disorder. These data suggest that the C1494T mutation is a novel mtDNA mutation that causes a genetic predisposition to aminoglycoise-induced and non-syndromic deafness.

**Biochemical defects caused by a combination of the deafness-linked mitochondrial tRNA<sup>Ser</sup>(UCN) mutation with two mtDNA mutations lead to the high penetrance of deafness in a large African American pedigree.** *X. Li<sup>1</sup>, N. Fischel-Ghodsian<sup>2</sup>, R. Friedman<sup>3</sup>, M.X. Guan<sup>1</sup>*. 1) Div Human Genetics, Children's Hosp Medical Ctr, Cincinnati, OH; 2) Ahmanson Department of Pediatrics, Cedars-Sinai Medical Center, Los Angeles, California 90048; 3) House Ear Clinic and House Ear Institute, Los Angeles, California 90057.

Mutations in the mitochondrial DNA (mtDNA) have been shown to be one of important causes of deafness. Recently, an African-American family with maternally inherited nonsyndromic hearing loss has been associated with the mitochondrial tRNA<sup>Ser</sup>(UCN) T7511C mutation, which has been found in genetically unrelated pedigrees with non-syndromic deafness. In addition, the homoplasmic mutations T3308C in the ND1 gene and T5655C in the tRNA<sup>Ala</sup> gene have been found in all members of this pedigree and also in some controls. We report here an investigation of pathogenetic mechanism underlying these mtDNA mutations by analyzing three mutant and three control transformants, constructed by transferring mitochondria from lymphoblastoid cell lines derived from a deaf individual and one control individual, into human mtDNA-less (o) cells. We show that the T7511C mutation leads to the decrease (~75%) in the amount of tRNA<sup>Ser</sup>(UCN) but not co-transcribed ND6 mRNA, which in mutant cells. Furthermore, the T5655C mutation yields the reduction (~50%) in the tRNA<sup>Ala</sup> level in these mutant cells. Strikingly, the T3308C mutation causes a significant decrease both in the amount of ND1 mRNA and adjacent tRNA<sup>Leu</sup>(UUR) in mutant cell lines. A combination of these alternations leads to the significant biochemical defects in the rate of mitochondrial protein synthesis, substrate-dependent respiration and the rate of growth in medium containing galactose in mutant cell lines. These observations provide the first direct biochemical evidence that three mtDNA mutations in different genes contribute to the high penetrance of non-syndromic deafness in the African American family.

**Genomic convergence: Identification of candidate genes for Parkinson Disease (PD) using genetic linkage and gene expression in the substantia nigra.** *M.A. Hauser<sup>1</sup>, M. Nouredine<sup>1</sup>, J.W. Walter<sup>1</sup>, R.W. Walters<sup>1</sup>, C.M. Hulette<sup>1</sup>, D.E. Schmechel<sup>1</sup>, M.L. Bembe<sup>1</sup>, J.M. Stajich<sup>1</sup>, H. Xu<sup>1</sup>, E.R. Martin<sup>1</sup>, B.L. Scott<sup>1</sup>, J. Stenger<sup>1</sup>, Y.J. Li<sup>1</sup>, R. Jensen<sup>3</sup>, C. Scherzer<sup>2</sup>, S. Gullans<sup>2</sup>, J.M. Vance<sup>1</sup>.* 1) Duke Univ Medical Ctr, Durham, NC; 2) Brigham and Womens Hospital, Harvard Medical School, Boston, MA; 3) Department of Physics, Wesleyan University.

Parkinsons Disease (PD) is a progressive neurodegenerative disease characterized by the loss of dopaminergic neurons in the substantia nigra. We are investigating candidate susceptibility genes for PD using Genomic Convergence, a multifactorial approach that combines linkage, expression analysis, SNP association and other methods. We have previously reported five large genomic linkage peaks in 174 multiplex PD families. We present here gene expression profiling in the substantia nigra of 6 patients with PD, 2 with progressive supranuclear palsy (PSP), one with frontotemporal dementia with Parkinsonism (FTDP), and 5 controls. PSP and FTDP are related parkinsonisms that, like PD, show loss of dopaminergic neurons. This allows us to assess the effect of changes in cell population within the PD samples. All samples were analyzed with Affymetrix U133A microarrays, and a subset was analyzed by serial analysis of gene expression. Genes from several metabolic pathways are significantly ( $p < 0.05$ ) altered in PD patients. The two molecular chaperones HSPA1A and HSPA1B are overexpressed. Apoptosis is suppressed: levels of the pro-apoptotic mediators NGFRAP1, MOAP1, and MTCH1 are reduced, while the level of the anti-apoptotic Bcl-2 is increased. There are reductions in vesicle transport genes: 9 subunits of the vacuolar ATPase ATP6 are reduced, as are GDI1, STXB1, SYT1, and VAMP1, proteins involved in docking of secretory vesicles. A large number of nuclear encoded mitochondrial proteins are also downregulated in PD patients. These genes (and others in the same pathways) that map to regions of PD linkage are being investigated as susceptibility genes: polymorphic variants identified by sequence analysis and family-based association analysis of tightly-linked polymorphisms will be presented.

**Identification of genes important in hypothalamic regulation of body weight.** *R. Wevrick, M.A. O'Neill, J.M. Bischof.* Department of Medical Genetics, University of Alberta, Edmonton, Alberta, Canada.

Obesity is one of the major health issues facing the developed countries today. Despite years of population studies, the genetic determinants of healthy body weight are poorly understood. Likewise, although there is increased understanding of the role of the hypothalamic-pituitary-adrenal axis in obesity, the proteins implicated in the development and/or function of this organ system are not all known. Specific proteins, such as hormones and their receptors, are responsible for control of appetite and long term maintenance of body weight. An increasing number of studies rely on mapping obesity modifier loci in mice or humans, then choosing candidate genes within the mapped region for further study. Still other studies examine changes in gene expression in mice undergoing dietary challenges, or in obesity gene knockout versus wild type mice. The hypothalamus is a complex region of the brain involved in homeostatic processes including, but not limited to energy balance. We have examined the relative expression levels of an estimated 30,000 genes in the murine hypothalamus as determined by Affymetrix microarray technology and made available by the Novartis Research Foundation (<http://expression.gnf.org>). From this, we identified a set genes not previously recognized to be important in hypothalamic function, but highly and preferentially expressed in the murine hypothalamus. We used bioinformatic databases to map the selected genes to chromosomal regions in the mouse genome and human genomes. We correlated the mapped positions of selected genes with the genomic positions of published loci influencing obesity related traits. We are now experimentally determining the developmental time frame for expression in specific murine hypothalamic nuclei with distinct functions in appetite and homeostasis, using northern blot and RNA in situ hybridization. These proteins can become targets for pharmacological intervention in obese individuals. Since susceptibility to obesity is in part genetically determined, we may also determine that variations in these genes contribute to familial obesity.

**Genetic Evaluation of Protein Tyrosine Phosphatase-1B (PTP-1B) Gene Polymorphisms and Measures of Glucose Homeostasis: The IRAS Family Study.** *N.D. Palmer<sup>1</sup>, C.D. Langefeld<sup>2</sup>, W.M. Brown<sup>2</sup>, F. Hsu<sup>2</sup>, D.W. Bowden<sup>1</sup>.* 1) The Center for Human Genomics, Wake Forest University, Winston-Salem, NC; 2) Public Health Sciences, Wake Forest University, Winston-Salem, NC.

PTP-1B is a ubiquitously expressed protein that dephosphorylates proteins at tyrosyl residues and has been shown to modulate the activity of the insulin receptor and its substrates. We have evaluated 24 SNPs in a 161kb region encompassing the ten exons spanning 74kb of PTP-1B. SNPs were genotyped on 1152 Hispanic (San Antonio, TX and San Luis Valley, CO) and African American (Los Angeles, CA) participants in the Insulin Resistance Atherosclerosis Family Study. To test for association among each PTP-1B SNP and trait, a series of generalized estimating equations was computed. A sandwich estimator of the variance and exchangeable correlation was used to account for familial correlation. SNPs within a region of 86kb, containing a portion of PTP-1B, were found to be in high linkage disequilibrium ( $D > 0.7$ ). Within this LD block, multiple SNPs show statistically significant associations with measures of glucose homeostasis. In combined population analysis, significant associations were with fasting glucose, insulin sensitivity (Si), and acute insulin response (AIR) ( $P = < 0.001 - 0.045$ ). In population specific analyses, the San Antonio population showed associations to Si, disposition index, and fasting glucose ( $P = < 0.001 - 0.050$ ), the San Luis Valley population to fasting glucose and Si ( $P = < 0.001 - 0.043$ ), and the Los Angeles population to AIR ( $P = 0.003 - 0.035$ ). Measures of adiposity were also evaluated for association. In combined populations analyses, visceral and subcutaneous adipose tissue, measured by computed tomography, were significantly associated ( $P = 0.002 - 0.012$ ). In center specific analyses, the San Luis Valley and Los Angeles populations showed associations to waist and waist to hip ratio ( $P = < 0.001 - 0.050$ ). In conclusion, PTP-1B polymorphisms are associated with measures of glucose homeostasis and have moderate, but significant associations with adiposity measures in Hispanic and African American populations.

**Amino acid substitutions underlying Mendelian disease are different from those implicated in complex diseases.**  
*P.D. Thomas*<sup>1</sup>, *A. Kejariwal*<sup>1</sup>, *D.A. Gilbert*<sup>2</sup>, *J.J. Sninsky*<sup>3</sup>. 1) Protein Informatics, Celera Genomics, Foster City, CA; 2) Applied Biosystems, Foster City, CA; 3) Celera Diagnostics, Alameda, CA.

We have recently developed a method for quantifying the severity of amino acid substitutions, based on an evolutionary conservation score (Thomas *et al.*, *Genome Research*, in press). In brief, the method uses an alignment of evolutionarily-related protein sequences and statistics from Hidden Markov Models, to estimate the probability that a given substitution at a given position will affect the proteins function. In other words, whether a substitution is conservative or radical will depend not only on the amino acids involved, but *where* in the protein it occurs. We have shown that these position-specific scores are much better than amino acid difference scores, such as the Grantham scale, at distinguishing known (mostly Mendelian) disease-causing alleles from normal variation. Here, we apply this method to analyzing amino acid substitutions (cSNPs) that are believed to play causative roles in Mendelian and complex diseases. We show that Mendelian disease cSNPs have a very strong tendency to occur in the most highly conserved regions of proteins, suggesting that they generally have a severe impact on the molecular-level function of the protein. Putative causative cSNPs underlying complex diseases, however, have a significantly weaker tendency to occur at conserved sites. In fact, the distribution of evolutionary conservation scores for complex disease cSNPs is much more similar to the distribution for normal human variation, than it is to the distribution for Mendelian disease cSNPs. Although there are relatively few generally accepted cSNPs implicated in complex disease, the difference from Mendelian disease cSNPs is statistically significant with  $P < 10^{-6}$ . This suggests that caution should be exercised when using Mendelian disease as a model for complex disease, at least with respect to molecular-level effects on protein function.

**The spectrum and frequency of MEFV, TNFRSF1A, and NALP3/CIAS1/PYPAF1 mutations in patients with AA amyloidosis associated with systemic inflammation.** *E. Aganna<sup>1</sup>, P.N. Hawkins<sup>2</sup>, H.J. Lachmann<sup>2</sup>, A. Bybee<sup>2</sup>, L. Karenko<sup>3</sup>, T. Pettersson<sup>3</sup>, A. Ranki<sup>3</sup>, G.A. Hitman<sup>1</sup>, P. Woo<sup>4</sup>, M.F. McDermott<sup>1</sup>.* 1) Unit of Molecular Medicine, Barts and London, UK; 2) Centre for Amyloidosis, Royal Free, UK; 3) Dept. of Medicine, Helsinki University, Finland; 4) Rheumatology, University College, London, UK.

**Objective:** We have investigated for the presence of mutations in the MEFV, TNFRSF1A, and NALP3/CIAS1/PYPAF1 genes in patients suffering from recurrent inflammatory attacks with and without AA amyloidosis. **Methods:** Genomic DNA from 233 unrelated patients with recurrent inflammatory attacks due to variety of conditions [juvenile idiopathic arthritis (JIA), rheumatoid arthritis (RA), Crohn's disease and periodic fever syndromes] were analysed. Mutational screening was done using the Transgenomic DHPLC (WAVE) system with subsequent sequencing of samples using an ABI 3100 automated sequencer and standard restriction fragment length analysis. **Results:** Four of 67 RA patients with amyloidosis had MEFV gene variants; of these 3 had E148Q, one being homozygous and two heterozygous; the fourth FMF patient had the V726A mutation, which is one of the five common mutations in FMF. It was also notable that in a TRAPS family (4 affected members with C88Y TNFRSF1A mutation) the single patient with amyloidosis was the only individual with E148Q. Two JIA patients with amyloidosis were found to have an R92Q variant of TNFRSF1A. A novel C to G transition in exon 2 of this gene, producing a histidine to glutamine at residue 22 (H22Q), was identified in a patient with JIA without AA amyloidosis. The reduced penetrance V200M variant of NALP3/CIAS1/PYPAF1, was identified in one FMF patient with amyloidosis and in an RA patient without amyloidosis. **Conclusion:** We have identified two MEFV gene variants in 67 RA patients with amyloidosis but none in 34 RA patients without amyloidosis. The R92Q TNFRSF1A variant was found in 2 of 61 JIA patients with AA amyloidosis but not in 80 controls. Mutations in the MEFV and TNFRSF1A genes may play a role in the development of AA amyloidosis in RA and JIA patients and E148Q increases the risk of this complication in TRAPS patients.

**Association analysis of VDR, -2-Macroglobulin, TNFR11, FcR111A and ACE gene polymorphisms in South Asian Rheumatoid Arthritis patients of the East Midlands, UK.** A. Ghelani<sup>1</sup>, A. Pacynko<sup>1</sup>, A. Gilmour<sup>1</sup>, L. Goh<sup>2</sup>, A. Jones<sup>3</sup>, A. Samanta<sup>2</sup>, J.I. Robinson<sup>4</sup>, A.W. Morgan<sup>4</sup>, J.D. Isaacs<sup>5</sup>, S.S. Mastana<sup>1</sup>. 1) Human Sciences, Loughborough University, Loughborough,UK; 2) Leicester Royal Infirmary, Leicester, UK; 3) City Hospital, Nottingham,UK; 4) St. James' University Hospital, Leeds,UK; 5) University Medical School, Newcastle Upon Tyne, UK.

**Introduction:** Rheumatoid Arthritis (RA) is a polygenic disease characterised by localised joint destruction. The pathogenesis of RA is complex but genetic factors such as Vitamin D receptor (VDR) gene which regulates the bone turnover and mass, Alpha-2-macroglobulin (A2M) involved in modulation of cytokines may play a significant role. Tumor necrosis factor RII (TNFR11) is associated with tissue destruction. FcR111A is linked with the clearance of immune complexes and Angiotensin-converting enzyme (ACE) is expressed in the synovial membranes during angiogenesis. We have analysed VDR, A2M, TNFR11, FcR111A and ACE genotypes in the development of RA amongst South Asians. **Methods:** DNA samples from 132 South Asian RA patients and 142 matched controls were analysed for VDR, A2M, TNFR11, FcR111A and ACE loci. The association analysis was performed by computing Odds ratios and Chi-square values ( $P < 0.05$ ). **Results:** Our results indicate an increased frequency of BB genotype of VDR gene in patients compared to controls leading to highly significant differences ( $\chi^2 = 24.70$ , DF 2,  $P < 0.01$ ). Similarly, A2M 22 genotype was prominent in patients ( $\chi^2 = 10.61$ , DF 2,  $P < 0.01$ ). TNFR11(GG) and FcR111A (VV) genotypes were slightly higher in patients but not did attain statistical significance. The ACE I/D polymorphism was not associated with RA in the present sample. The BB genotype of VDR locus (Odds ratio = 3.89) and A2M 22 genotype (Odds ratio = 4.18) were associated with RA. **Conclusions:** Our preliminary analyses suggests that the VDR BB and A2M 22 genotypes were associated with RA among South Asians. The mechanism by which the VDR polymorphism is associated with RA is unknown, but they could be related to immuno-regulatory properties of vitamin D. A2M association may be due to cytokine binding affinity which could modulate the progression of RA.

**A Candidate Gene for Idiopathic Scoliosis.** *S. Bashiardes*<sup>1</sup>, *R. Veile*<sup>1</sup>, *C.A. Wise*<sup>2</sup>, *L. Szappanos*<sup>2</sup>, *M. Lovett*<sup>1</sup>. 1) Genetics, Washington University, St. Louis, MO; 2) Texas Scottish Rite Hospital for Children, Dallas, TX.

Idiopathic scoliosis (IS) affects approximately 1-2% of the population. The genetics of this disorder are complex and several loci have been suggested with genome-wide linkage scans. Here we describe a family in which a pericentric inversion of chromosome 8 co-segregates with idiopathic scoliosis. It is possible that scoliosis was caused by disruption of a gene at one of these breakpoints. To further characterize these regions we used fluorescent in situ hybridization (FISH) to identify cloned DNAs that span the breakpoints on both chromosomal arms. We identified a BAC of 150kb that crosses the q arm breakpoint and a BAC of 120kb that crosses the p arm breakpoint. The complete genomic DNA sequences of these BACs were analyzed to identify candidate genes and to further localize the precise breakpoint. This revealed that the p-arm breakpoint does not appear to disrupt any known gene and occurs in a region of highly repetitive sequence elements. On the q-arm, the break occurs between exons 10 and 11 of the gamma-1 syntrophin (SNTG1) gene. Syntrophins are a group of cytoplasmic peripheral membrane proteins that associate directly with dystrophin, the Duchenne muscular dystrophy gene. Dystrophin acts as a protein complex scaffold and different members of the syntrophin family bind dystrophin depending on the type of tissue. Gamma- 1 syntrophin has been shown to be a neuronal cell specific protein. Preliminary analysis of 152 sporadic IS patients revealed a 6 base deletion in exon 10 of SNTG1 in one patient and a single base pair mutation occurring in a polypyrimidine tract of intronic sequence 20 bases upstream of the SNTG1 exon 5 splice site in another patient. These changes were not seen in a screen of 240 control chromosomes. At present, it is unclear whether alterations to SNTG1 co-segregate with scoliosis in any familial cases of IS.

**Smoking status and Estrogen Receptor genotype show significant interaction with Bone Mineral Density in the Framingham Offspring Study.** A. Shearman<sup>1</sup>, D. Karasik<sup>2</sup>, L.A. Cupples<sup>3</sup>, S. Coven<sup>1</sup>, K. Gruenthal<sup>1</sup>, D.E. Housman<sup>1</sup>, D.P. Kiel<sup>2</sup>. 1) Center Cancer Res, Massachusetts Inst Technology, Cambridge, MA; 2) Hebrew Rehab Ctr for Aged & Harvard Med Sch, Boston, MA; 3) Biostatistics, BU Sch of Public Health, Boston, MA.

There are conflicting results from studies of estrogen receptor alpha (ESR1) polymorphisms and bone mineral density (BMD). Smoking affects estrogen metabolism, is associated with lower BMD and greater risk of osteoporosis, and may affect ESR1 transcription. We hypothesized that smoking might influence the association between ESR1 and BMD. We studied 732 men and 792 women (mean age 60 yrs). BMD was measured by DXA at the hip and lumbar spine. Covariates: age, height, BMI, current cigarette smoking (12% of men and 15% of women), and estrogen use in women. Six common ESR1 polymorphisms were genotyped. We used ANOVA/ANCOVA to estimate the main effects of each polymorphism, as well as haplotype probabilities derived using the expectation-maximization algorithm, on crude and covariate-adjusted BMD in each sex separately. No significant main associations between ESR1 polymorphisms and BMD were observed. In contrast, significant interactions between PvuII and XbaI genotypes, smoking, and BMD ( $p = 0.004$  to  $0.01$ ) were found in men, but not women. PvuII and XbaI are in strong LD and in men interactions were also found between smoking, common haplotypes, and BMD at the hip. A substantial additive effect of combined PvuII and XbaI genotypes was found only among men who smoked: BMD was higher in pp-xx compared to PP-XX, difference of 12% (femoral neck), 14% (trochanter), 20% (Wards area), and 9% (lumbar spine). Conclusion: common Estrogen Receptor polymorphisms are associated with BMD in men who smoke. This highlights the important role of estrogen in men, and more generally of environmental interactions and the need for studies large enough to identify them.

**Linkage of Reading Disabilities and Attention-Deficit Hyperactivity Disorder to the Chromosome 15q Region.** C.

*Barr*<sup>1,2</sup>, *Y. Feng*<sup>1</sup>, *T. Pathare*<sup>2</sup>, *B. Anderson*<sup>2</sup>, *T. Cate-Carter*<sup>2</sup>, *J. Crosbie*<sup>2</sup>, *M. Malone*<sup>2</sup>, *W. Roberts*<sup>2</sup>, *R. Tannock*<sup>2</sup>, *A. Ickowicz*<sup>2</sup>, *R. Schachar*<sup>2</sup>, *M. Lovett*<sup>2</sup>, *T. Humphries*<sup>2</sup>, *J. Kennedy*<sup>3</sup>, *K. Wigg*<sup>1</sup>. 1) Toronto Western Hospital; 2) The Hospital for Sick Children; 3) The Centre for Addiction and Mental Health, Toronto, ON, Canada.

Twin studies support an overlap in the heritabilities for specific reading disabilities (RD) and attention-deficit hyperactivity disorder (ADHD), with 95% of the overlap for inattention symptoms and RD due to common genetic influences. Linkage findings have been reported for several regions for RD (1p, 2p, 6p, 6q, 15q, 18p) with the 6p and 15q regions reported to be linked to the ADHD phenotype in families with RD. We investigated the overlap of these phenotypes in the 15q region using two samples of nuclear families, one ascertained through a proband with reading difficulties (n=118 families) and the other through a proband with ADHD (n=186 families). Based on previous studies of this region, four microsatellite markers, D15S214, D15S994, D15S146 and D15S143, were chosen to be genotyped in these families. Using the transmission disequilibrium test (TDT) we found evidence for biased transmission of alleles of the marker D15S146 in the ADHD sample for the ADHD phenotype as a categorical trait (chi-square =6.5, 1d.f., p=0.01) and in the RD sample for the categorical trait of RD (chi-square=5.4, 1 d.f., p=0.02). The D15S146 marker is located in an intron of a putative G-protein coupled receptor gene (GPR). To further investigate this region, we genotyped 3 SNPs in the GPR locus, flanking the D15S146 marker. Haplotype analysis of these markers in the ADHD sample showed one haplotype with biased transmission to affected children (chi-square=4.6, 1d.f., p=0.03). Using a quantitative approach to look at evidence for linkage to the reading phenotypes of phonological awareness, decoding, and word identification we observed evidence for linkage to one of the GPR markers in the RD sample. Our study provides evidence for a susceptibility locus contributing to both RD and ADHD in the 15q region. Further the finding of allelic association for the D15S146 and GPR markers, substantially narrows the region in which this gene resides.

**Molecular characterization of chromosome 4p inversion breakpoints in two autistic siblings.** *S. Choufani*<sup>1</sup>, *J. Vincent*<sup>1, 3</sup>, *J. Skaug*<sup>1</sup>, *M. Li*<sup>1</sup>, *D.A. Kwasnicka*<sup>1</sup>, *B.A. Fernandez*<sup>4</sup>, *P. Szatmari*<sup>5</sup>, *W. Roberts*<sup>2</sup>, *S.W. Scherer*<sup>1</sup>. 1) Department of Genetics and Genomic Biology; 2) The Child development Center, The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Neurogenetics Section, Centre for Addiction and Mental Health, Toronto, On; 4) Discipline of Genetics, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Nf; 5) Canadian centre for Studies of Children at Risk, Hamilton, On, Canada.

Autism is a severe neurodevelopmental disorder, characterized by impairments in social interaction and communication skills. Chromosomal abnormalities among autistic individuals have been described, and include almost all chromosomes. We describe here two brothers diagnosed with autism and having paracentric inversion of the short arm of chromosome 4 [46, XY, inv(4) (p12-p15.3)]. It was determined that this inversion was inherited from an apparently unaffected mother. Bacterial artificial chromosome (BAC) clones spanning the breakpoints were identified by means of fluorescence in situ hybridization (FISH) analysis. The distal p15.3 breakpoint was present between D4S3036 and D4S2906. A region downstream to this (12Mb) was linked to autism at markers D4S2936 and D4S412 using ASPEX with a multipoint (MLS) of 1.55 (IMGSA, 1998). The proximal breakpoint was between D4S1536 and D4S396 in a region previously shown to be disrupted in a familiar case of autism in a girl having dup (4) p12-p13 (Sabaratnam M. et al., 2000). The proximal breakpoint (4p12) maps to a region containing a cluster of the gamma-aminobutyric acid A (GABA(A)) receptor genes. This region is of particular interest because elevated plasma GABA levels have been reported in autistic children suggesting a role for GABA as biochemical marker for autistic disorder. Furthermore, another cluster of GABA(A) receptor genes are located in the 15q11-q13 candidate autism region. Our findings provide further support for the possible involvement of the short arm of chromosome 4 in the genetic etiology of autism. We are currently assessing all candidate genes in the vicinity of the breakpoints by sequencing DNA samples from autistic multiplex families.

**GENETIC STUDY OF PARAOXONASE GENE VARIANTS IN AUTISTIC DISORDER.** *M. D'Amelio*<sup>1</sup>, *T.H. Wassink*<sup>2</sup>, *L. Muscarella*<sup>3</sup>, *V. Guarnieri*<sup>3</sup>, *L. D'Agruma*<sup>3</sup>, *L. Zelante*<sup>3</sup>, *R. Militerni*<sup>4</sup>, *S. Trillo*<sup>5</sup>, *F. Montecchi*<sup>5</sup>, *M. Elia*<sup>6</sup>, *C. Schneider*<sup>7</sup>, *R. Melmed*<sup>7</sup>, *T. Pascucci*<sup>8</sup>, *S. Puglisi-Allegra*<sup>8</sup>, *K.L. Reichelt*<sup>9</sup>, *J. Piven*<sup>2</sup>, *A.M. Persico*<sup>1</sup>. 1) Lab. of Mol. Psychiatry & Neurogenet, Univ. Campus Bio-Medico, Rome, Italy; 2) Dept of Psychiatry, Univ. of Iowa Sch. of Med., Iowa City, IA and N.D.R.C., Univ. of North Carolina, Chapel Hill, NC; 3) Med. Genet. Serv., IRCCS Casa Sollievo dalla Sofferenza, S.Giovanni Rotondo (FG), Italy; 4) Child Neuropsychiatry, II Univ. of Naples, Italy; 5) Child Neuropsychiatry, IRCCS Osp. Bambino-Ges, Rome, Italy; 6) Neurology Serv., IRCCS Oasi Maria S.S., Troina (EN), Italy; 7) S.A.R.C., Phoenix, AZ; 8) Dept. of Psychology, Univ. La Sapienza, Rome, Italy; 9) Dept. of Pediatric Res., Rikshospitalet, Univ. of Oslo, Norway.

We have described an association, replicated in at least two independent studies, between autism and long alleles of a polymorphic GGC repeat located in the 5'UTR of the gene encoding Reelin, a pivotal protein for neuronal migration during neurodevelopment. We have also found that long GGC alleles yield reduced protein translation rates, both in vitro and in vivo, and that Reelin exerts a proteolytic activity potently inhibited by organophosphates (OP), compounds routinely used as pesticides and insecticides. Within the framework of a gene-environment interactive model, genetically-vulnerable individuals producing lower amounts of Reelin, if prenatally exposed to OP during critical periods in neurodevelopment, could undergo altered neuronal migration resulting in an autistic syndrome. Paraoxonase, the enzyme responsible for OP detoxification, displays impressive 40-fold interindividual differences in humans, largely resulting from functional polymorphisms present in the paraoxonase (PON1) gene, located on human ch 7q21.3. Current studies carried out on 263 complete trios with primary autistic probands using functional SNPs located in the promoter and coding sequence of the PON1 gene yield significant linkage/association (Q192R TDT  $\chi^2 = 4.85$ , 1df,  $P < 0.05$ ) in Caucasian-American and not in Italian families, as predicted on the basis of our prior Reelin findings. Supported by Telethon-Italy (GPP02019), the Fondation Jerome Lejeune, and the CAN/AGRE.

**Ghrelin and Peptide YY Levels and Gene Expression in Prader-Willi Syndrome.** *M.G. Butler, Z. Talebizadeh, D.C. Bittel.* Children's Mercy Hospitals and Clinics and University of Missouri-Kansas City School of Medicine, Kansas City, MO.

Prader-Willi syndrome (PWS) is due to imprinting caused by a deficiency in expression of paternal genes on chromosome 15q11-q13 through a deletion (Del) or UPD. One of the main hallmarks of PWS is an insatiable appetite leading to early childhood obesity. An important objective of our study is to better understand the abnormalities in appetite-regulating pathways in PWS including at the gene level. Ghrelin and peptide YY are produced by the gastrointestinal system and involved with controlling appetite. We obtained fasting plasma ghrelin and peptide YY (PYY) levels in 12 PWS infants and children ranging in age from 2.5 months to 13.3 years and six healthy control subjects ranging in age from 5 to 43 years. Ghrelin levels in our PWS children and infants were similar to other PWS subjects and were significantly higher (3 to 5X) than reported in obese children without PWS. Interestingly, the PYY levels in our PWS group were lower than reported in similarly aged subjects without PWS. It is unclear why ghrelin levels are high and peptide YY levels are low in subjects with PWS. The genes for ghrelin, peptide YY and their receptors (i.e., GHS-Ra1, GHS-Rb1 and NPY2) are not localized on chromosome 15q. We performed preliminary gene expression studies using quantitative RT-PCR on 4 of these subjects [1 control (15-yrs-old male) and 1 subject from each PWS genetic subtype (1-yr-old female Del; 1.5-yrs-old female UPD; and 13-yrs-old male IC)]. Expression level of ghrelin, GHS-Ra1, GHS-Rb1, PYY and NPY2 were measured in RNA extracted from lymphoblast cell lines. GAPDH, a housekeeping gene, was used to normalize the expression data. All five genes showed higher expression levels (e.g., 2X) in the control than PWS subjects. In addition, the UPD and deletion subjects had markedly lower expression level than the PWS subject with an imprinting defect. The expression levels of these genes will be measured in additional subjects (controls and PWS). Expression studies with the five genes were also performed in frontal cortex from our 1-yr-old female Del subject and will be compared with other regions of the brain as well as brain tissue from control subjects.

**Genetic variants in a haplotype block spanning *IDE* influence plasma A42 levels and risk for Alzheimers disease.**

*N. Ertekin-Taner*<sup>1,2</sup>, *M. Allen*<sup>2</sup>, *D. Fadale*<sup>2</sup>, *L. Scanlin*<sup>2</sup>, *L. Younkin*<sup>2</sup>, *R.C. Petersen*<sup>3</sup>, *N. Graff-Radford*<sup>4</sup>, *S.G. Younkin*<sup>2</sup>.

1) Dept Neuroscience, Mayo Clinic, Rochester, MN; 2) Dept Neuroscience, Mayo Clinic, Jacksonville, FL; 3) Dept Neurology, Mayo Clinic, Rochester, MN; 4) Dept Neurology, Mayo Clinic, Jacksonville, FL.

Risk for late onset Alzheimers disease (LOAD) and plasma A42 levels, an intermediate phenotype for LOAD, show linkage to chromosome 10q. Several strong candidate genes (*VR22*, *PLAU*, *IDE*) lie within the 1-lod support interval for linkage. Two groups independently identified haplotypes in the chromosome 10q region harboring *IDE* that show highly significant association with intermediate AD phenotypes and with risk for AD. To pursue these associations, we analyzed the same haplotypes for association with plasma A42 in 24 extended LOAD families and for association with LOAD in two independent case-control series. One series (MCR, 183 age-matched case-control pairs) did not show association ( $p=0.43$ ) with the six haplotypes in the 276 kb region spanning three genes (*IDE*, *KNSL1*, and *HHEX*), previously shown to associate with LOAD. The other series (MCJ, 93 age-matched case-control pairs) showed highly significant ( $p<10^{-7}$ ) association with these haplotypes. In the MCJ series, the H4 (OR=5.1,  $p=0.001$ ) and H5(H10) haplotypes (OR=0.45,  $p=0.06$ ) had the same effects previously reported. In this series, the H8 haplotype (OR=5.3,  $p<0.001$ ) also had a significant effect as in one previous case control series but not in others. In the extended families, the H8 haplotype was associated with significantly elevated plasma A42 ( $p=0.02$ ) and the H5(H10) haplotype with reduced plasma A42 ( $p=0.007$ ). These results provide strong evidence for pathogenic variant(s) in the 276 kb region harboring *IDE* that influence intermediate AD phenotypes and risk for AD.

Support Contributed By: AG18023 (SGY), AG06656 (SGY), AG20903 (NET), American Federation for Aging Research Grant PD01062 (NET) and a Robert and Clarice Smith Fellowship (NET).

**Fine mapping of the -T Catenin gene to a quantitative trait locus on chromosome 10 in late-onset Alzheimers**

**disease pedigrees.** *M. Carrasquillo*<sup>1</sup>, *N. Ertekin-Taner*<sup>1,2</sup>, *J. Ronald*<sup>1</sup>, *H. Asahara*<sup>1</sup>, *L. Younkin*<sup>1</sup>, *M. Hella*<sup>1</sup>, *S. Jain*<sup>1</sup>, *E. Gnida*<sup>1</sup>, *S. Younkin*<sup>1</sup>, *D. Fadale*<sup>1</sup>, *Y. Ohyagi*<sup>3</sup>, *A. Singleton*<sup>1</sup>, *L. Scanlin*<sup>1</sup>, *M. deAndrade*<sup>4</sup>, *R. Petersen*<sup>5</sup>, *N. Graff-Radford*<sup>6</sup>, *M. Hutton*<sup>1</sup>, *S.G. Younkin*<sup>1</sup>. 1) Department of Neuroscience, Mayo Clinic, Jacksonville, FL; 2) Department of Neuroscience, Mayo Clinic, Rochester, MN; 3) Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; 4) Department of Health Sciences Research, Division of Biostatistics, Mayo Clinic, Rochester, MN; 5) Department of Neurology, Mayo Clinic, Rochester, MN; 6) Department of Neurology, Mayo Clinic, Jacksonville, FL, USA.

Using plasma amyloid protein (A42) levels as an intermediate, quantitative phenotype for late onset Alzheimers disease (LOAD), we previously obtained significant linkage at 80 centimorgans (cM) on chromosome 10. Linkage to the same region was obtained independently in a study of affected LOAD sib-pairs. Together, these two studies provide strong evidence for a novel LOAD locus on chromosome 10 that acts to increase A42. *VR22* is a large (1.7 Mb) gene located at 80 cM that encodes -T catenin, which is a binding partner of catenin. This makes *VR22* an attractive candidate gene because catenin interacts with presenilin 1, which has many mutations that elevate A42 and cause early onset familial AD. We identified two intronic *VR22* SNPs (4360 and 4783) in strong linkage disequilibrium (LD) that showed highly significant association ( $p=0.0001$  and  $0.0006$ ) with plasma A42 in 10 extended LOAD families. This association clearly contributed to the linkage at 80 cM because the lod scores decreased when linkage analysis was performed conditional upon the *VR22* association. The association was robust because it replicated in two independent sets of LOAD families. Bounding of the association region using multiple SNPs showed *VR22* to be the only confirmed gene within the region of association. These findings indicate that *VR22* has variant(s) which influence A42 and contribute to the previously reported linkage for plasma A42 in LOAD families.

**NON-ALLELIC GENETIC HETEROGENEITY IN AUTOSOMAL DOMINANT LATERAL TEMPORAL LOBE EPILEPSY AND ABSENCE OF LGI1 MUTATIONS IN FAMILIAL MESIAL TEMPORAL LOBE**

**EPILEPSY.** A. Badhwar<sup>1</sup>, L. Racacho<sup>3</sup>, D. D'Agostino<sup>1</sup>, F. Dubeau<sup>1</sup>, F. Andermann<sup>1</sup>, D. Bulman<sup>3</sup>, E. Andermann<sup>1, 2</sup>. 1) Dept Neurology & Neurosurg, Montreal Neurological Hospital and Institute, McGill Univ, Montreal, PQ, Canada; 2) Dept Human Genetics, McGill Univ; 3) Ottawa Health Research Institute, Ottawa, Ontario, Canada.

Familial temporal lobe epilepsy (FTLE) is subdivided into two groups: familial mesial temporal lobe epilepsy (FMTLE) and autosomal dominant lateral temporal lobe epilepsy (ADLTLE) with auditory features. Mutations in LGI1 (chr10q23) were identified in several ADLTLE families. Since we found occasional individuals with auditory features in FMTLE families, we evaluated 21 FTLE patients for mutations in LGI1, postulating either pleiotropy or allelic heterogeneity of these two conditions. Detailed clinical and family histories were obtained in 18 FTLE families. 21 patients (18 probands) were screened for mutations in the LGI1 gene. Each exon and the surrounding splice sites were amplified by PCR and sequenced directly. In total, 8 exons were sequenced as well as the entire intron between exons 3 and 4. Mean age of onset was 15.7 years, ranging from infancy to 52 years. 20% had a history of febrile convulsions, 13% had *deja-vu*. 40% had intractable seizures, and 13% had been operated successfully. Seizure types included simple partial, complex partial and occasional secondary generalization. Three probands were the only ones in their families with auditory hallucinations. 56% showed hippocampal atrophy and 33% had hippocampal gliosis. There was an average of 3.7 relatives with epilepsy per family, most with temporal lobe epilepsy. No mutations of the LGI1 gene were identified. We identified two known and one novel SNP. The lack of mutations in LGI1 in our families suggests that the molecular basis of FMTLE differs from that in ADLTLE. Furthermore, absence of LGI1 mutations in our patients with auditory features demonstrates non-allelic heterogeneity in ADLTLE.

**Variant in calcium-sensing receptor promoter region is associated with Alzheimer disease status.** *Y. Conley*<sup>1,2</sup>, *H. Brockway*<sup>1</sup>, *D. Finegold*<sup>2</sup>, *R. Ferrell*<sup>2</sup>. 1) Health Promotion & Development; 2) Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

The calcium-sensing receptor (CASR) is primarily involved with systemic calcium ion homeostasis, although its systemic function does not explain why it is expressed in brain tissue. We previously reported the association of an intronic CASR polymorphism with Alzheimer disease (AD) status. We believed that this polymorphism was likely to be in linkage disequilibrium with another variant in or around the CASR gene and began screening the coding region, intron/exon boundaries, and promoter region of the CASR gene for a variant that could have a functional impact on the CASR. Using dHPLC we located a previously unreported variant in the CASR promoter region. It is located in the second promoter, 176nt away from the alternative transcriptional start site. We genotyped a subset of our AD cases and controls for the variant, and detected a significant association between the variant and AD status. Genotype and allele frequencies differed significantly ( $p < 0.001$ ) between AD cases ( $n=274$ ) and age-matched, race-matched, geographically matched and non-demented controls ( $n=162$ ). Of note is that this variant is only 8nt away from a vitamin D response element. Experiments are currently in progress to assess this variant for functionality, postulating that it could alter level of expression of the CASR gene product.

**Deletion of the endogenous mouse Snca gene markedly exacerbates synucleinopathy in transgenic mice expressing Ala53->Thr.** *D.E. Cabin*<sup>1</sup>, *S. Gispert*<sup>2</sup>, *D. Murphy*<sup>3</sup>, *G. Auburger*<sup>5</sup>, *R. Myers*<sup>4</sup>, *R.L. Nussbaum*<sup>1</sup>. 1) NHGRI/GDRB, NIH, Bethesda, MD; 2) Institute for Experimental Neurobiology University Frankfurt/M, Germany; 3) National Institute for Neurological Disease and Stroke, NIH, Bethesda, MD; 4) University of California at San Diego, San Diego, CA; 5) Section Molecular Neurogenetics, Bldg 26, University Hospital, Frankfurt/M, Germany.

Mutations in  $\alpha$ -synuclein (SNCA) are known to cause Parkinsons disease in a subset of familial cases. The normal function of SNCA is unknown, but it is a component of Lewy bodies, the neuronal inclusions diagnostic for Parkinsons disease. An A53T human SNCA transgene under the control of the mouse prion promoter was crossed onto mice that lack endogenous Snca. Though the expression level of the mutant transgene is low, knockout mice expressing only the human mutant SNCA develop a fast-progressing limb weakness and paralysis with the earliest age of onset at 16 months and peaking at about 19 months of age. This is probably caused by inappropriate expression of A53T SNCA in the spinal cord, particularly in the ventral region, leading to Wallerian degeneration in the ventral roots. However, mice carrying the same transgene on a wild type background only rarely develop the neuropathy and only at a later age. This may be due to some protective effect of the mouse protein, though endogenous levels are low in the ventral spinal cord and roots. At position 53, the wild type mouse amino acid is the same as the human mutation, indicating that mice are either too short-lived to suffer ill effects from the threonine, or else have developed compensatory changes elsewhere in Snca or in other proteins. Our results shows that the best approach to generating good mouse models for Parkinsons disease will be to use human transgenes controlled by a promoter that does not drive spinal cord expression in mice that lack endogenous Snca.

**Association Study Of Dopamine D2 Receptor (DR2) and Bipolar Affective Disorder in Colombian Population.** C. Duran<sup>1</sup>, J.C. Prieto<sup>1,2</sup>, A. Ordonez<sup>1</sup>, J.F. Ortiz<sup>3</sup>, D. Patermina<sup>3</sup>, R. Tamayo<sup>3</sup>, F. Rivadeneira<sup>3</sup>, C. Gomez<sup>3</sup>. 1) Instituto de Genetica Humana, Universidad Javeriana., Bogota, Cundinamarca, Colombia; 2) Hospital la Victoria, SDS, Bogota, Colombia; 3) Hospital San Ignacio, Departamento Psiquiatria, Bogota, Colombia.

Bipolar affective disorder (BPD) is a major psychiatric disorder that affects 1% of the population worldwide and it is characterized by episodes of mania and depression. The studies suggesting the dopamine D2 receptor (DRD2) gene as a candidate gene for schizophrenia includes the correlation of the antipsychotic potency of neuroleptic drugs with their affinity for the receptor. In Japanese was found the variant Cys 311, produced by a substitution of serine with cysteine (Ser 311-Cys) at codon 311 of the receptor. The objective of this study is to investigate association of the variant Cys 311 of the dopamine D2 receptor gene. We have performed a case-control analysis in 62 cases with bipolar affective disorder and 81 control subjects. Allele frequencies for Ser311 and Cys 311 alleles were similar in the control subjects Ser311=0.975, Cys311=0.025 and cases Ser311=0.984 and Cys311=0.016. No statistically significant difference in the allele frequencies (chi square 0.241, p=0.623) was observed between cases and control likewise we found no evidence of association of this polymorphism with BPD in this sample. Our results suggest other genes involving in BPD in our population.

**Frequency of the Glucocerebrosidase N370S Mutation and Susceptibility to Parkinson's Disease in a Cohort of Ashkenazi Jewish Ethnicity.** *L.N. Clark<sup>1, 2</sup>, A. Nicolai<sup>2</sup>, J. Harris<sup>4</sup>, H. Mejia-Santana<sup>3</sup>, L.J. Cote<sup>3, 4</sup>, E.D. Louis<sup>3, 4</sup>, S. Fahn<sup>3</sup>, K. Marder<sup>1, 3, 4</sup>.* 1) Taub Institute for Research on Alzheimer's Disease and the Aging Brain; 2) Department of Pathology; 3) Gertrude H. Sergievsky Center; 4) Department of Neurology, Columbia University, New York, NY.

Mutations in the glucocerebrosidase gene located at chromosome 1q21 are associated with Gaucher Disease (MIM 230800), a glycolipid storage disorder that is clinically heterogeneous presenting with a wide spectrum of phenotypes, including neuronopathic and non-neuronopathic forms. Recent studies suggest that a deficiency in glucocerebrosidase associated with a milder form of late-onset Type I Gaucher disease may contribute to a susceptibility to Parkinson's disease. One of the most common mutations reported in Ashkenazi Jewish patients with Type I Gaucher disease is N370S with the gene frequency for this allele estimated to be 0.031 in this population. In one study, Tayebi et al (2003) showed that 82% of patients with parkinsonian symptoms had at least one N370S mutant allele (all six Ashkenazi Jewish patients included in the study had one N370S allele with 4 patients homozygous for N370S), and although brain glucosylsphingosine levels were not elevated a loss of pigmented neurons in the substantia nigra and Lewy bodies were observed in post mortem tissue available for four probands. To determine the frequency and relative genetic contribution of N370S to Parkinson's disease in patients of Ashkenazi Jewish ethnicity we have determined genotypes in a large clinically well-characterized Ashkenazi Jewish cohort of 192 PD probands that includes 59 early-onset cases (mean age at onset 43.6 years) and 133 late-onset cases (mean age at onset 65.5 years) that were ascertained independent of family history in addition to controls matched by gender, education and ethnicity. PD cases were recruited from the Center for Parkinson's Disease and Other Movement Disorders (CPD) at the College of Physicians and Surgeons at Columbia University. We report the frequency of the N370S Glucocerebrosidase mutation in both early-onset and late-onset Parkinson's disease cases of Ashkenazi Jewish ethnicity.

**Gene expression of APOE allele specific hippocampal tissues in Alzheimers disease.** *J.R. Gilbert<sup>1</sup>, Y-J. Li<sup>1</sup>, P-T. Xu<sup>1</sup>, X.J. Qin<sup>1</sup>, D. Schmechel<sup>3</sup>, C. Hulette<sup>3</sup>, A. McLaurin<sup>1</sup>, C. Sherzer<sup>4</sup>, S. Gullans<sup>4</sup>, J. Haines<sup>2</sup>, M.A. Pericak-Vance<sup>1</sup>.* 1) Center for Human Genetics, Duke Univ, Durham, NC; 2) PHG, Vanderbilt Univ. Nashville, TN; 3) ADRC, Duke Univ. Med, Ctr, Durham, NC; 4) Brigham and Womens Hospital, Boston, MA.

Total RNA was isolated from the hippocampi of 12 Alzheimer disease (AD) patients and 3 age matched controls. AD patients were of APOE4/4, APOE4/3, and APOE3/3 genotypes and matched for Braak and Braak (B&B) stages IV and V and post mortem delay. Controls were APOE3/3 (B&B stage I). Gene expression in these samples was assayed using Affymetrix GeneChips (U133A) microarrays containing 22,000 predicted or known human genes. Gene expression analysis showed significant differential expression between AD hippocampus and controls, and between APOE 4/4 and 3/3 AD samples, but not between B&B stage IV and V AD tissues. For analysis genes with average raw sample signal intensity of less than 500 were excluded from statistical analysis. After statistical filtering 1541 genes remained for analysis. A p value of 0.05 was used as a cut-off for significance. The methods of Benjamini and Hochberg were used to estimate the false discovery rate (FDR). A two-sample t-test identified 237 significant differentially expressed genes between AD and controls by p-values, with 124 genes showing significant results by FDR. For the comparison of B&B stages IV and V within AD samples, 48 genes demonstrated significant differential expression by p-values, but were excluded after FDR correction, implying that gene expression patterns do not significantly change between B&B stages IV and V. The APOE genotype of AD patients did have an effect on gene expression with 99 genes showing significant expression differences by the p-value and 27 genes after FDR correction between APOE 4/4 and APOE 3/3 of AD samples. This study will identify differentially expressed genes between AD and control individuals as well as significant affected pathways and potential candidate genes for further study. These data will also be integrated with our ongoing genetic mapping studies (genomic convergence) to identify high priority candidate genes for AD genetic risk.

Program Nr: 2001 from 2003 ASHG Annual Meeting

**Possible association between haplotype of the GABA-A receptor alpha 1 subunit gene (GABRA1) and mood disorders.** *Y. Horiuchi<sup>1</sup>, J. Nakayama<sup>1</sup>, H. Ishiguro<sup>1</sup>, T. Ohtsuki<sup>1</sup>, T. Toyota<sup>2</sup>, K. Yamada<sup>2</sup>, M. Nankai<sup>3</sup>, H. Shibuya<sup>4</sup>, T. Yoshikawa<sup>2</sup>, T. Arinami<sup>1</sup>.* 1) Dept Medical Genetics, Univ Tsukuba, Tsukuba, Japan; 2) Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, Wako, Saitama, Japan; 3) Tokyo Metropolitan Police Hospital, Department of Neuropsychiatry, Tokyo, Japan; 4) National Sanatorium Minami-Hanamaki Hospital, Hanamaki, Iwate, Japan.

The gamma-aminobutyric acid (GABA) neurotransmitter system has been implicated in the pathogenesis of mood disorders. The GABRA1 gene encodes one of the subunits of GABA-A receptors and is located on human chromosome 5q34-q35, which is one of the regions reported to be linked to mood disorders. We examined the GABRA1 gene as a candidate for mood disorders. We performed mutation screening of the GABRA1 in 24 Japanese bipolar patients and evaluated associations in Japanese case-control subjects consisting of 125 bipolar patients, 147 patients with depressive disorders, and 192 healthy controls. We identified 13 polymorphisms in the GABRA1 gene. Associations of the specific haplotype with affective disorders was suggested in the Japanese case-control population ( $p=0.001$ ) These results indicate that the GABRA1 gene may play a role in the etiology of bipolar disorders.

**Cytogenetic and molecular characterization of a chromosome 3q inversion in a patient with language delay. D.A.**

*Kwasnicka*<sup>1</sup>, *A.M. Summers*<sup>2</sup>, *Y. Qi*<sup>1</sup>, *W. Roberts*<sup>3</sup>, *S. Choufani*<sup>1</sup>, *S.W. Scherer*<sup>1</sup>. 1) Department of Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Genetics, North York General Hospital, Toronto, Ontario, Canada; 3) Department of Pediatrics, The Child Developmental Center, Toronto, Ontario, Canada.

We describe a paracentric inversion of chromosome 3 (inv(3)(q25-29)) in a 5-year-old girl with developmental delay, delayed expressive, and receptive language. Haplotype data on the family suggest that the chromosomal origin of the inversion is inherited from the father, who is also language delayed but socially normal. The aim of the study is to characterize the q25-q29 chromosomal breakpoints in order to identify candidate genes associated with the phenotype. Bacterial artificial chromosome (BAC) clones spanning the breakpoints were identified using fluorescence in situ hybridization (FISH). The proximal and distal breakpoints were found to be located between genetic markers D3S2404/D3S1553 and D3S3642/D3S2305, respectively. Results from a genome scan in a genetically isolated multiplex Finnish families has recently provided evidence for a new autism susceptibility locus (AUTS2) on the chromosome 3q25-27 (Auranen et al., 2002). This might be relevant to our study since speech and language disorder is a common component of the autism phenotype. We are, therefore, working to refine the precise site of the inversion breakpoints to identify candidate genes for testing in additional patients with language disorder, as well as those who fall within the autism-spectrum.

**DNA pooling identifies association of the noradrenergic transporter gene (NET1) with attention deficit hyperactivity disorder (ADHD).** *J. Knight, X. Xu, K. Brookes, J. Mill, P. Sham, E. Taylor, P. Asherson.* SGDP, Institute of Psychiatry, KCL, London, United Kingdom.

This study set out to identify associations between ADHD NET1. The noradrenergic system is known to play a role in attentional mechanisms and is thought to be important in ADHD. Stimulant medications are known to alter the activity of noradrenaline as well as dopamine in the synapse and the highly selective noradrenergic reuptake inhibitor, atomoxetine, is an effective treatment for ADHD symptoms. To date replicated associations have been described with several genes that regulate monoamine neurotransmission (DRD4, DAT1, SNAP-25, DRD5 and 5HT1B). Here we set out to investigate whether common polymorphisms within NET1 are associated with the DSM-IV ADHD combined subtype, using a sample that has previously shown association with genes that regulate synaptic release and reuptake of neurotransmitters (DAT1 and SNAP-25). We identified all SNPs from publicly available databases that have minor allele frequencies 10 % and span the NET1 genomic region (n=35). Heterozygosity rates were validated before association analysis (n=21 in final set). DNA pooling was used to screen for associations (n=180 cases, n=334 controls) and positive findings confirmed by individual genotyping. DNA was quantified for pooling by fluometry and allele frequency estimates obtained using a primer extension method (Snapshot). We identified 6 SNPs that showed significant allele frequency differences between the case and control pools (rs2242447 p=0.002; rs998424 p=0.00004; rs3785157 p=0.0007; rs747107 p=0.037; rs42460 p=0.046; rs2242446 p=0.049). Preliminary analysis of individual genotypes and multi-marker haplotypes support the initial DNA pooling results but do not provide additional evidence of association. Assuming these data reflect a true association, this suggests that a functional variant conferring risk for ADHD is likely to be in strong linkage disequilibrium with, or may indeed be, one of the three markers showing the highest evidence for association. Further work aims to confirm the association using within-family tests, identify likely functional variants and test for functionality.

**Association between nicotine dependence and an A/G polymorphism in the beta-muscular nicotinic acetylcholine receptor (CHRNA1) gene.** *J.Z. Ma<sup>1</sup>, J. Beuten<sup>1</sup>, R.T. Dupont<sup>2</sup>, K.M. Crews<sup>3</sup>, T.J. Payne<sup>3</sup>, M.D. Li<sup>1</sup>.* 1) The University of Texas Health Science Center, San Antonio, TX; 2) The University of Tennessee Health Science Center, Memphis, TN; 3) The University of Mississippi Medical Center, Jackson, MS.

Epidemiological studies have strongly implicated that genetics play a significant role in the determination of smoking behavior and nicotine dependence; however, the susceptibility genes responsible for these phenotypes remain to be characterized. Previously, our genome-wide linkage analysis indicated that the 17p13.1 chromosomal region might harbor candidate gene(s) for nicotine dependence. One of the genes located in this region is the beta-muscular nicotinic acetylcholine receptor gene which represents a potential candidate for influencing smoking behavior and nicotine dependence. Several single nucleotide polymorphisms within the CHRNA1 gene have been identified and two of them were assayed for association with smoking behavior in this study by using the 5' nuclease allelic discrimination technique in both Caucasian and African-American populations. Association analysis using the ASSOC program of the S.A.G.E. package suggested that there exists a strong association between an A/G polymorphism in the CHRNA1 gene and the number of cigarettes smoked per day, as well as and the Fagerstrom Test for Nicotine Dependence (FTND) score, in the African-American population under both the additive and dominance genetic model ( $P < 0.03$ ). However, no significant association was obtained between this A/G polymorphism and both phenotypes under the additive, dominance, or recessive model in the Caucasian population. These results suggest that this A/G polymorphism in the CHRNA1 gene may be related to nicotine dependence in the African-American population (supported by DA-12844).

Program Nr: 2005 from 2003 ASHG Annual Meeting

**Fine-mapping of a Chromosome 7 substance abuse vulnerability locus implicates NrCAM gene regulatory regions.** *H. Ishiguro, Q. Liu, G.R. Uhl.* Dept Molecular Neurobiology, NIDA/NIH, Baltimore, MD.

Classical genetic studies demonstrate substantial heritabilities for alcoholism and drug abuse, with most of the genetic vulnerabilities being shared between these two disorders. COGA consortium studies displayed alcoholism links to a broad chromosome 7 region centered on D7S1793 and D7S1830. Previous studies in our laboratory demonstrated association of SNP markers in this region with polysubstance abuse in each of two samples of NIDA research volunteers. To pursue fine mapping of this locus, I have examined 60 SSLP markers in this region in polysubstance abusers and control individuals. A cluster of chromosome 7 97-103 Mb markers revealed nominally-significant associations with substance abuse. In our lab, a neuronal cell adhesion molecule gene in this region (NrCAM) was previously defined as morphine-regulated, and as expressed in dopaminergic brain regions that are important for the rewarding effects of most if not all abused substances. Cocaine- and amphetamine-induced NrCAM regulation in mice brain are also identified, as well as receptor type protein tyrosine phosphatase beta (RPTPb), one of homologous or heterologous binding partners. Interactions between NrCAM and RPTPbeta could well influence the development or maintenance of brain circuits important for acute drug reward. We thus produced a sequence alignment and haplotype definitions in Caucasian and African-American samples in the human NrCAM genomic region, based on genotypes at SSLPs and SNPs. The genomic region associated with drug abuse and alcoholism contained several blocks of restricted haplotype diversity, and haplotypes that were associated with addiction vulnerability in several different populations. Initial studies of mRNAs from the brains of individuals heterozygous for these haplotypes reveal different levels of expression of NrCAM mRNAs from these two haplotypes. Allelic/haplotypic analysis for the RPTPbeta also suggests association with substance abuse. Convergent data from molecular genetic and neurobiological approaches thus strongly supports NrCAM, and possibly its binding partners, as important genes for human addiction vulnerability.

**7q Candidate Gene screening in Autistic disorder.** *M. Mori<sup>1</sup>, T. Yamagata<sup>1</sup>, H. Li<sup>1</sup>, T. Goto<sup>1</sup>, K. Suwa<sup>1</sup>, A. Yasuhara<sup>2</sup>, M.Y. Momoi<sup>1</sup>.* 1) Department of Pediatrics, Jichi Medical School, Tochigi, Japan; 2) Department of Pediatrics, Kouri Hospital, Kansai Medical University, Osaka, Japan.

Although many candidate gene loci were reported and a strong genetic component was indicated in autistic disorder, only a few diseases related genes were identified. Among these loci, Chromosome 7q is a strong candidate gene locus of autistic disorder. There are some positional-functional candidate genes such as FOXP2, Wnt family gene, small G-protein-coupled receptor genes on chromosome 7q. We previously analyzed and reported some candidate genes such as FOXP2 and HTR5a. This time we analyzed Wnt family genes and small G-protein-coupled receptor genes on chromosome 7q. Patients diagnosed as pervasive developmental disorder or autism according to the criteria of DSM-were enrolled in this study after informed consents by their parents. 70 Japanese patients and 100 American patients (from AGRE) were analyzed. This study was approved by the bioethics committee for human gene analysis at Jichi Medical School. Genomic DNA was extracted from the peripheral blood leukocytes using the standard methods. All exons of each gene were amplified separately by PCR. Mutations were screened by DHPLC analysis with WAVE DNA-fragment analysis system (Transgenomic, Inc., Omaha). And mutations were confirmed by direct sequencing analysis. We found several polymorphisms and two missense mutations in Wnt family gene and small G-protein-coupled receptor gene, respectively. One missense mutation in WNT gene was detected also in his unaffected mother, but not in 134 normal individuals. It seemed that these mutations had a possibility of causing disease, although they were not conclusive. Because these genes were expressed especially in the brain, these mutations would induce some changes in higher brain functions. Further screening of candidate genes in larger sample set and study on the functional change of identified mutation as well as candidate gene knockout animal model analysis is needed to clarify their contribution to the autistic disorder.

**Apolipoprotein E SNP haplotypes: association with hemorrhagic stroke.** R. Kaushal<sup>1</sup>, D. Woo<sup>2</sup>, M. Haverbush<sup>2</sup>, P. Pal<sup>1</sup>, N. Wang<sup>1</sup>, J. Khoury<sup>1</sup>, P. Shekar<sup>2</sup>, B. Kissela<sup>2</sup>, C. Moomaw<sup>2</sup>, L. Sauerbeck<sup>2</sup>, G. Sun<sup>1</sup>, R. Chakraborty<sup>1</sup>, J. Broderick<sup>2</sup>, R. DeKa<sup>1</sup>. 1) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Dept Neurology, Univ of Cincinnati, Cincinnati, OH.

Stroke is the foremost cause of disability and the third leading cause of death in the western hemisphere. It is one of the most complex diseases with several subtypes as well as secondary risk factors such as hypertension, hyperlipidemia, and diabetes, which in turn have genetic and environmental risk factors of their own. Variations in the apolipoprotein E (*APOE*) gene have been associated with Alzheimers disease which shares a common pathophysiologic mechanism (amyloid deposition) with lobar intracerebral hemorrhage (ICH). The genetic heterogeneity of *APOE* has also been associated with aneurysmal subarachnoid hemorrhage (SAH) as well as outcome following stroke. However, these associations remain inconclusive. To test for association, we genotyped four SNP markers in the *APOE* gene (*rs1160983*, *rs1160984*, *rs1160985* and *rs157581*), spanning a distance of 11 kb, in 98 cases with SAH and 181 age, gender, and race-matched controls and 105 cases of ICH and 179 matched controls. Haplotype analysis revealed significant differences in SAH cases and controls. Further, stratification based on race (Caucasians and African Americans) showed even greater differences between Caucasian cases of SAH and controls (p 0.001). Of the observed 10 haplotypes, three, viz., 1111, 1211, 1212 (1 = wild, 2 = mutant allele), showed significant differences. The ICH group was further sub-divided into lobar versus non-lobar cases. There was a significant difference between cases and controls of lobar ICH for haplotypes 1211 (cases - 28%, controls - 47%; p = 0.03) and 2212 (cases - 6%, controls - 0%; p = 0.04). In the same group, the marker *rs157581* also showed significant differences in allele frequencies between cases and controls (allele 1 = 80% in cases, and 63% in controls; p = 0.024). These results indicate that *APOE* gene plays a significant role in both subarachnoid hemorrhage and lobar intracerebral hemorrhage. Supported by NIH grant NS36695.

**Parkin mutations cause late-onset slowly progressing Parkinson's disease.** *J. Harris<sup>1</sup>, S. Afridi<sup>2</sup>, P. Greene<sup>1</sup>, S. Fahn<sup>1</sup>, K. Marder<sup>1, 2, 3</sup>, L.N. Clark<sup>2, 4</sup>.* 1) Department of Neurology,; 2) Taub Institute for Research on Alzheimer disease and the Aging Brain,; 3) Gertrude H. Sergievsky Center, and; 4) Department of Pathology, Columbia University, New York, NY.

The Parkin gene on chromosome 6q25.2-27 is associated with autosomal recessive, juvenile-onset Parkinson's disease (PD) and sporadic young-onset PD (age of onset <45 years). Slower progression of disease and a good response to levodopa have been reported as characteristic clinical features of young-onset patients with Parkin mutations. More recently, homozygous and heterozygous mutations have been identified in the Parkin gene in familial and sporadic late-onset PD. We hypothesized that late-onset PD patients with clinical symptoms resembling young-onset Parkin-related PD may have a higher prevalence of Parkin mutations. Thus, we selected late-onset PD patients (>45 years), with features of the characteristic Parkin phenotype including slow progression and good response to levodopa. Patients (n=22) were selected who continued to have mild symptoms in the medicated state after years of symptoms. The mean age at onset of our group of patients was 61.4 years (range 48-76 years) and the mean duration of symptoms at time of last evaluation was 12.4 years (range 5-25 years). The patients were selected blind to family history. Sequencing and gene dosage analysis of the Parkin gene in cases and matched controls was performed. We report the frequency of Parkin mutations and polymorphisms in these patients and in a matched control series. There was no significant difference in positive family history between our patients with and without Parkin mutations. We found that a significant percentage of our patients with slow progression, good response to levodopa and onset >45 years have Parkin mutations. This suggests that Parkin mutations may be much more common in late-onset PD than previously reported.

**Investigation of Candidate Genes in Pathological Gambling.** *M.M. Krinsky<sup>1</sup>, P.M. Muglia<sup>1,2</sup>, J.L. Kennedy<sup>1,2</sup>, N.E. Turner<sup>3</sup>.* 1) Neurogenetics Section, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; 2) Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada; 3) Department of Social Health and Policy, Centre for Addiction and Mental Health, Toronto, Ontario, Canada.

Pathological gambling (PG), according to the DSM-IV, is an impulse-control disorder that is characterized by persistent and maladaptive gambling behaviors. A recent meta-analysis reported the lifetime prevalence of PG in adults at 1.6%, with an additional 3.9% of adults having a gambling problem (Shaffer, 1999). Family, twin, and genetic association studies have reported that the susceptibility to PG has a relatively large genetic component. Like other psychiatric conditions, PG is considered a complex disorder since it is likely determined by several genes interacting together as well as with environmental factors. Converging lines of evidence have suggested the dopaminergic pathways represent important principal biological components involved in the etiology of PG. We collected DNAs on 145 gambling subjects (55 PG, 31 borderline-PG, and 55 non-PG) who underwent a thorough assessment including DSM-IV criteria and the South Oaks Gambling Screen. We genotyped 5 dopamine-related candidate genes (DRD4, DAT, COMT, DRD5 and TH) and compared the phenotype scores across genotype groups. We were unable to detect a significant association with any of the polymorphisms genotyped (DRD4:  $t=-0.501$ ,  $p=0.617$ ; DAT:  $t=1.251$ ,  $p=0.213$ ; COMT:  $t=-1.639$ ,  $p=0.104$ ; DRD5:  $t=-0.404$ ,  $p=0.687$ ; TH:  $t=0.695$ ,  $p=0.489$ ) however, there was a trend for the biased presence of the associated risk allele in the PG group for DRD4 (7-repeat) 42% and COMT (allele 2) 23%. Furthermore, we observed a higher frequency of the risk allele of the DRD4 (7-repeat), COMT (Allele 2) and DRD5 (148 bp) genes than in previously reported studies. Correlation studies showed that the additive genotypic effects of the candidate genes (excluding TH) accounted for 2.9% of variance in our sample ( $r=0.171$ ,  $p=0.046$ ). These results are preliminary and further analyses are underway using additional candidate genes, larger samples and matched case-control subjects.

**Association of SNPs in Dopaminergic Pathway Genes with young onset and late onset Parkinson's Disease among Indians.** *B.K. Thelma*<sup>1</sup>, *S. Punia*<sup>1</sup>, *M. Behari*<sup>2</sup>, *U. Muthane*<sup>3</sup>, *R.C. Juyal*<sup>4</sup>. 1) Dept Zoology, Univ Delhi, Delhi, India; 2) Dept Neurology, All India Institute of Medical Sciences, New Delhi, India; 3) Dept of Neurology, National Inst. of mental health and neurosciences, Bangalore, India; 4) National Inst. of Immunology, New Delhi, India.

Parkinson's disease is a progressive neurodegenerative disorder characterized by bradykinesia, resting tremor, muscular rigidity, postural instability and has a prevalence of >1% in 55-year old individuals and >3% in those >75 years of age. Among Indian PD patients, ~10% and 20% are accounted for, by young onset and familial PD respectively and a large majority falls under the late onset category. Mutations in genes such as alpha Synuclein, Parkin, DJ-1, UCHL-1, have been observed in some familial forms of PD. However, the genetic basis of a large majority of young onset as well as the late onset PD cases is still not clear. Various candidate genes from the dopaminergic pathway, cytochrome P450 family of genes, oxidative stress genes and mitochondrial genes have been implicated for development of PD by various mechanisms. SNPs have emerged as powerful markers for establishing association of candidate chromosomal region(s) with complex traits. We have, in this study undertaken to establish, association if any, of a large number of SNPs in some candidate genes from the dopaminergic pathway with young onset PD (n=180) and late onset PD (n=345) using a case-control approach. Significant associations of SNP(s) in the tyrosine hydroxylase, dopamine receptor D2, and catechol O-methyltransferase genes have been observed in both young onset and late onset PD in our study.

**Evidence supporting *CENTG2* as an autism susceptibility gene.** *T.H. Wassink<sup>1</sup>, J. Piven<sup>2</sup>, V.J. Vieland<sup>1,3</sup>, L. Jenkins<sup>1</sup>, R. Goedken<sup>3</sup>, M.A. Spence<sup>4</sup>, M. Smith<sup>4</sup>, V.C. Sheffield<sup>5</sup>.* 1) Department of Psychiatry, University of Iowa Carver College of Medicine, Iowa City, IA; 2) Neurodevelopmental Disorders Research Center and Department of Psychiatry, University of North Carolina, Chapel Hill, NC; 3) Department of Biostatistics, University of Iowa College of Public Health, Iowa City, IA; 4) Department of Pediatrics, University of California, Irvine, Irvine, CA; 5) Department of Pediatrics and the Howard Hughes Medical Institute, University of Iowa Carver College of Medicine, Iowa City, IA.

We identified three autistic subjects with 2q37.3 terminal deletions, and refined the deletion breakpoint regions using polymorphism mapping and FISH probes. These breakpoints, and others in the literature, were within or immediately upstream of the gene centaurin gamma-2 (*CENTG2*). While numerous genes are absent in these deletions, *CENTG2* is an attractive candidate autism disease gene. It is expressed in the developing brain and is involved in biomolecular pathways that influence cellular functions such as axon growth and guidance, cellular differentiation, metabolism, and vesicular trafficking. We therefore assessed *CENTG2* for its involvement in autism by 1) screening its exons for variants in 135 unrelated autistic individuals and 160 control subjects, and 2) assessing for linkage and LD 6 intra-genic polymorphisms in 60 autism ASP families. The exon screen revealed a Ser to Gly substitution in one proband, an Arg to Gly substitution in another, both in conserved functional domains; a CT transition in two sibs that significantly diminished the strength of a potential exon splice enhancer, a common Ile to Val substitution, and an intronic AG deletion in one father and his two affected children that may lead to aberrant splicing of exon 12. We also identified an intriguing mini-satellite polymorphism in intron 1 the predominant and secondary alleles are 1110 and 660 nt long, respectively that is in linkage with autism. In addition, strong evidence for linkage emerged with a maximum two-point HLOD value of 4.2 from a polymorphism in intron 10; no evidence for LD was identified at any of the markers tested. We conclude, therefore, that *CENTG2* may contribute to autism susceptibility.

**Association of GSK-3 polymorphisms with Alzheimer's disease and frontotemporal dementia.** *B.A.J. Schaffer<sup>1</sup>, B.L. Miller<sup>2</sup>, K.C. Wilhelmsen<sup>3</sup>, S. Weintraub<sup>4</sup>, N. Johnson<sup>4</sup>, E. Bigio<sup>5</sup>, B. Quinn<sup>4</sup>, M.-M. Mesulam<sup>4</sup>, J.L. Cummings<sup>1</sup>, D. Masterman<sup>1</sup>, D.H. Geschwind<sup>1</sup>.* 1) Dept. of Neurology, David Geffen School of Medicine, UCLA, Los Angeles, CA; 2) Dept. of Neurology, UCSF, San Francisco, CA; 3) Gallo Clinic and Research Ctr., San Francisco, CA; 4) Depts. of Neurology and Psychiatry & Behavioral Sciences, Northwestern Univ. Feinberg School of Medicine, Chicago, IL; 5) Dept. of Pathology, Northwestern Univ. Feinberg School of Medicine, Chicago, IL.

Pathologic phosphorylation of the microtubule associated protein tau is a hallmark of many neurodegenerative diseases, including Alzheimer's disease (AD), frontotemporal dementia (FTD), primary progressive aphasia (PPA) and progressive supranuclear palsy (PSP). Thus, genes that phosphorylate tau are logical candidate genes for these diseases. We have previously shown that GSK-3 induced hyperphosphorylation of tau is an important mediator of neurodegeneration in a drosophila model and here explore its potential association with AD and FTD.

We sequenced the promoter of GSK-3 and all 12 exons including surrounding intronic sequence in a mostly Caucasian population of FTD, AD and aged normal subjects. One new SNP was found in the promoter, one in exon 4, two in exon 9, and 11 in the introns. The rare variants occurring within exons did not change coded amino acids. However, a more common intronic polymorphism occurring with an allele frequency of 5.9% in aged normals (6/102 chromosomes) occurs at more than twice the frequency among FTD subjects (12.0%; 17/142 chromosomes) and AD subjects (13.8%; 13/94 chromosomes). This SNP is located 68 nucleotides upstream of exon 3 within an intronic region of high homology with mouse, suggesting that it plays an as yet unknown functional role. This study provides statistical support for association of polymorphisms within the GSK-3 gene and tau-related neurodegenerative conditions, including AD and FTD. This is the first genetic evidence that sequence variation within genes involved in tau phosphorylation may contribute to human neurodegenerative conditions.

Program Nr: 2013 from 2003 ASHG Annual Meeting

**Molecular genetic analysis of a chromosome 4q31.3-34 deletion associated with autism.** A. Woodroffe, S. Ramanathan, P. Flodman, M. Bocian, L. Mays, C. Modahl, M. Hanouni, M.A. Spence, M. Smith. Pediatrics, University of California- Irvine.

Autism is a developmental disorder characterized by impaired communication and social interactions and stereotypic patterns of interest and activity. This report is a case study of a subject who met ADI-R and ADOS-G diagnostic criteria for autism. He was also found to have significant language impairment, hypotonia, minor congenital anomalies, and an IQ of 59. The anomalies include metatarsus adductus, cryptorchidism, shortened metacarpals and syndactyly of the second and third toes. Through karyotyping, he was shown to have a deletion on one member of his chromosome 4 pair. We used FISH mapping and microsatellites to determine the breakpoints and extent of this deletion. Due to the lack of known polymorphic microsatellites in the region of interest, we developed 4 new polymorphic markers, two are located in the PDGFC gene, one in the glycoprotein M6A (GPM6A) gene, and one in the AMPA glutamate receptor A2 (GLUR2) gene. We found that the deletion extends about 19 Mb from 4q31.3-q34. Analysis revealed that the deletion occurred de novo, on the paternally derived chromosome. The telomeric end of the deletion is within the GPM6A gene, the centromeric end is within the PDGFC gene. 33 genes mapped to the deletion region, 14 of these genes are expressed in the brain. Five genes that are especially interesting are: GLUR2, the glycine receptors GLRA3 and GLRB, carboxypeptidase E (CPE) and GPM6A. The GLUR2 is of interest because glutamate receptor deficiency has been previously reported in autism. Also, Carlsson et al (1998) proposed that autism is a hypoglutamatergic disorder associated with decreased AMPA glutamate receptor response. Hemizygosity for CPE is interesting given its role in processing neuropeptides, including oxytocin, vasopressin, and proopiomelanocortin (POMC). The GPM6A gene encodes a neuronally expressed gene that plays a role in downstream nerve growth factor signaling pathway. The genes mentioned above, and other genes that map within the region corresponding to the deletion, constitute candidate genes for autism. [Supported by Grant NICHD HD35458 MA Spence PI].

**A missense coding polymorphism in the Urokinase-Type Plasminogen Activator gene (*PLAU*) is associated with Alzheimers disease and age-dependent changes in plasma A42 levels.** S.G. Younkin<sup>1</sup>, J. Ronald<sup>1</sup>, H. Asahara<sup>1</sup>, L. Feuk<sup>2</sup>, J. Prince<sup>2</sup>, M. Tucker<sup>3,4</sup>, L. Younkin<sup>1</sup>, M. Hella<sup>1</sup>, S. Jain<sup>1</sup>, L. Hersh<sup>4</sup>, M. Kindy<sup>4</sup>, W. Markesbery<sup>4</sup>, M. Hutton<sup>1</sup>, M. deAndrade<sup>5</sup>, R.C. Petersen<sup>6</sup>, N. Graff-Radford<sup>7</sup>, S. Estus<sup>3,4</sup>, A. Brookes<sup>2</sup>, N. Ertekin-Taner<sup>1,8</sup>. 1) Dept Neuroscience, Mayo Clinic, Jacksonville, FL; 2) Center for Genomics and Bioinformatics, Karolinska Institute, Stockholm, Sweden; 3) Dept Physiology, University of Kentucky, Lexington, KY; 4) Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY; 5) Dept Health Sciences Research, Mayo Clinic, Rochester, MN; 6) Dept Neurology, Mayo Clinic, Rochester, MN; 7) Dept Neurology, Mayo Clinic, Jacksonville, FL, USA; 8) Dept Neuroscience, Mayo Clinic, Rochester, MN.

Plasma amyloid protein (A42) levels and late onset Alzheimers disease (LOAD) have been linked to the same region on chromosome 10q. The *PLAU* gene, which is within this region, encodes urokinase-type plasminogen activator (uPA), which converts plasminogen to plasmin. *PLAU* is an attractive candidate because A aggregates induce *PLAU* expression thereby increasing plasmin, which degrades A. In this study, we analyzed a missense C/T polymorphism in exon 6 of *PLAU* (*PLAU\_1*) in which the minor T allele causes a proline to leucine change (P141L) within the kringle domain of *PLAU*. We analyzed *PLAU\_1* for association with AD in six case-control series by meta-analysis. The C/T and T/T *PLAU\_1* genotypes showed association with an overall estimated OR of 1.2. The C/T and T/T genotypes of *PLAU\_1* were also associated with significant, age-dependent elevation of plasma A42 in twenty-four extended LOAD families that we analyzed ( $p=0.0006$ ). In knockout mice lacking the *PLAU* gene, plasma A was significantly elevated, also in an age-dependent manner. The *PLAU\_1* associations reported here were independent of the associations we found with the genetic variants in the *IDE* or *VR22* regions. These results provide strong evidence that *PLAU* or a nearby gene is involved in the development of LOAD. *PLAU\_1* is a plausible pathogenic mutation that could act by increasing A42, but additional biological experiments are required to show this definitively.

**Association of FGF20 with late-onset Parkinson disease.** *J.M. van der Walt<sup>1,2</sup>, E.R. Martin<sup>1,2</sup>, W.K. Scott<sup>1,2</sup>, F. Zhang<sup>1,2</sup>, K. Fujiwara<sup>1,2</sup>, R. Kittappa<sup>3</sup>, M.A. Nance<sup>2</sup>, J.P. Hubble<sup>2</sup>, W.C. Koller<sup>2</sup>, R. Pahwa<sup>2</sup>, M. Stern<sup>2</sup>, B.C. Hiner<sup>2</sup>, J. Jankovic<sup>2</sup>, C.G. Goetz<sup>2</sup>, G.W. Small<sup>2</sup>, F. Mastaglia<sup>2</sup>, L.T. Middleton<sup>2</sup>, M.A. Pericak-Vance<sup>1,2</sup>, J.M. Vance<sup>1,2</sup>.* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Duke CHG/GlaxoSmithKline PD Genetics Collaboration; 3) National Institute of Health, National Institute of Neurological Disorders and Stroke, Bethesda, MD.

The pathogenic process responsible for the loss of dopaminergic neurons within the substantia nigra of Parkinson disease (PD) patients is poorly understood. However, there is strong evidence to support the involvement of fibroblast growth factor 20 (FGF20) in the survival of dopaminergic cells. FGF20 is a neurotrophic factor that is preferentially expressed within the substantia nigra of rat brain. The human homologue has been mapped to 8p21.3-8p22, which is also an area of PD linkage revealed through our published genomic screen. In order to test whether FGF20 influences risk of PD, we genotyped 3 single nucleotide polymorphisms (SNPs) using TaqMan in a large family based study. We analyzed our sample (N families = 440) using the pedigree disequilibrium test (PDT) and the genotype-PDT that assess association between alleles or genotypes, respectively, and risk. We revealed a highly significant association between an intronic SNP rs1989754 (p-value = 0.0006) and PD in our overall sample with and a weaker association with SNP rs1721100 (p-value = 0.02) located in a regulatory region. The frequency of allele rs1989754G is increased by 5% in the affected family member sibs relative to the unaffected family member sibs. Furthermore, genotype-PDT analysis revealed that genotype rs1989754GG is also positively associated with PD risk (p-value=0.02). We suggest that FGF20 may be a risk factor for late-onset PD. We are currently screening exons of FGF20 for variants that may affect the function of the protein.

**Linkage disequilibrium and haplotype analysis of BDNF gene variations in Parkinson's Disease.** A. Parsian<sup>1</sup>, R. Sinha<sup>1</sup>, B. Racette<sup>2</sup>, J.H. Zhao<sup>3</sup>, J.S. Perlmutter<sup>2</sup>. 1) Birth Defects Ctr, Univ Louisville Hlth Sci Ctr, Louisville, KY; 2) Department of Neurology, Washington University School of Medicine, St. Louis, MO; 3) Department of Epidemiology and Public Health, University College London, London, UK.

Most cases of Idiopathic Parkinson's disease (PD) are sporadic but approximately 20% have a positive family history suggesting a hereditary component. Although most of these 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. The goal of this study was to determine the role of the BDNF gene in the development of familial and sporadic PD. We sequenced the promoter region of the gene using genomic DNA from 45 patients with familial PD. 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 sample of 146 patients with and 212 without a positive family history for PD and 195 matched controls with the SNP at C270T and G196A in exon 5 of the gene. The 270T allele was more common in the familial PD subjects compared to normal controls ( $p=0.0006$ ) but not significantly different between sporadic PD and normal controls. The genotype frequencies were significantly different only between familial PD and normal controls ( $p=0.00001$ ). Both PD groups were categorized based on age of onset of 50 in two groups of  $<50$  and  $>50$  years. There was a highly significant difference in allele and genotype frequency between the familial group with age of onset of  $>50$  years and controls ( $p=0.0002$  and  $p=0.0001$ ). We estimated and compared the haplotype frequencies between C270T and G196A markers in PD and controls that was positive (Heterogeneity statistic was 14.9,  $p=0.0019$ ). Our data indicate the possibility of linkage disequilibrium between the C270T variation and a mutation in coding region of the BDNF gene and suggest that BDNF may play a role in the development of familial PD that warrant further study.

**Chromosome microdeletions as etiological factors in autism: Report of 4 cases and identification of autism candidate genes.** *M. Smith, P. Flodman, A. Woodroffe, M.E. Bocian, M. Hanouni, M.A. Spence.* Dept Pediatrics, Univ California, Irvine, Irvine, CA.

Autism is a complex neurodevelopmental disorder. Identification of autism candidate genes through linkage analysis is hampered because there are many more sporadic cases of autism than familial cases. Autism candidate genes may be identified through molecular genetic analysis of structural chromosome changes that occur in affected subjects. We have identified 4 autistic subjects who each have a different chromosome deletion. The following regions are involved: 15q22-q23, 2q37.3, 13q12-q13, 4q31.3-4q34. We carried out FISH studies and analyses of polymorphic microsatellite markers to define the deletion regions. Marker analysis revealed that each of the deletions arose de novo on a paternal chromosome. In consequence of deletion, patients were hemizygous for one or more brain expressed genes encoding molecules involved in neurodevelopment, signaling molecules or neurotransmitters. The 15q22-q23-deletion patient is hemizygous for Neogenin, a Netrin 1 receptor. Neogenin is expressed in developing forebrain, corpus callosum, hippocampus and dorsal thalamus. In consequence of the 2q37.3 deletion an autistic subject is hemizygous for centaurin gamma 2, a phosphatidyl inositol signaling pathway molecule. She is also hemizygous for neuronal homeobox gene GBX2 and ATSV axonal transporter of synaptic vesicles. The 13q12-q13 deletion leads to hemizyosity for DCAMKL1 that contains doublecortin and Cam kinase domains and plays a role in cortical development. He is also hemizygous for MADH9, a member of the SMAD TGF beta signaling pathway. Smad and Tgf beta mutants in *Drosophila* are associated with defective neuronal pruning. The 4q31.3-q34 deletion patient is hemizygous for 3 neurotransmitter receptor genes: AMPA glutamate receptor GLURA2 and the glycine receptor genes GLRA3, GLRB3. This subject is also hemizygous for carboxypeptidase E (CPE) that cleaves neuronal prepropeptides including oxytocin vasopressin and POMC. Chromosome changes are not uncommon in autism (Yu et al. AJHG 2002) comprehensive screening for them, using FISH, microsatellite and/or microarray analysis, is required.

**Association of a 5HT1B polymorphism with suicidal behavior.** *E.C. Tan<sup>1</sup>, Y.L. Tan<sup>2</sup>, Y.Y. Teo<sup>3</sup>, G. Lau<sup>4</sup>, C.H. Tan<sup>2</sup>.*  
1) Defense Medical Res Inst, Defense Science & Tech Agency, Singapore, Singapore; 2) Faculty of Medicine, National University of Singapore; 3) Department of Statistics, National University of Singapore; 4) Department of Pathology, Health Sciences Authority.

Personality traits which are highly heritable are often predictive of later development of psychiatric disorders and behaviors such as aggression, hostility, impulsivity and other anti-social behavior. Abnormalities in serotonergic neurotransmission have been implicated in suicidal ideation and behavior due to its function in mediating the control of mood, aggression and impulsivity. In particular, 5HT1B receptor knock-out mice showed increased aggression as well as increased alcohol and cocaine consumption. For suicide, a severe consequence of impulsive behavior, studies have found that the levels of some receptors such as the 5HT subtypes may be altered in brains from suicide victims and other disorders involving impulsive behavior. To test the hypothesis that the 5HT1B receptor mediated serotonergic dysfunction might be involved in suicidal behavior through the disinhibition of aggression and impulsivity, we examined the association of a single nucleotide polymorphism in the 5HT1B receptor gene and suicide. Our sample consisted of 333 confirmed suicide cases (mean age 43.6 SD = 19.1) and 110 controls (mean age 43.3 SD = 16.3) recruited from institute and hospital staff with informed consent. The HTR1B 861G>C polymorphism was genotyped by PCR-RFLP. There is statistically significant difference in genotype distribution between the control and suicide group ( $p = 0.02$ ). Subjects with at least one copy of G possess greater suicidal tendencies with the odds ratio at 1.97 (95% CI = 1.22,3.18). Testing for allelic effects with allele C as the baseline, the odds ratio for allele G is 1.41, 95% CI = (1.04,1.92). A gender effect on suicide tendencies is also observed ( $p = 0.01$ ) with our results showing that males are more at risk than females overall (odds ratio = 1.76, 95% CI = 1.14,2.72) although there are more female suicide victims among the young while the trend is reversed for the elderly. We would be expanding the sample size to increase the power of our study.

**DD genotype of the ACE gene is a risk factor for spontaneous intracerebral hemorrhage.** A. Slowik<sup>1</sup>, W. Turaj<sup>1</sup>, T. Dziedzic<sup>1</sup>, A. Haefele<sup>2</sup>, L. Sobanska<sup>1</sup>, P. Szerner<sup>1</sup>, J. Pera<sup>1</sup>, M. Malecki<sup>3</sup>, A. Szczudlik<sup>1</sup>, D.A. Figlewicz<sup>2,4</sup>. 1) Neurology, Jagiellonian University, Krakow, Poland; 2) Neurology, University of Rochester, Rochester, NY; 3) Metabolic Diseases, Jagiellonian University, Poland; 4) Neurology, University of Michigan, Ann Arbor, MI.

The angiotensin converting enzyme (ACE) gene is considered an important candidate gene for stroke involving small cerebral vessels, particularly spontaneous intracerebral hemorrhages (SIH) and ischemic strokes due to small vessel disease (SVD). An insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene has been shown to be associated with plasma ACE levels and activity. It accounts for approximately 45% of the total variability of serum ACE levels, with the highest values in DD allele carriers. The ACE enzyme plays a role in endothelial function, and the regulation of smooth muscle proliferation and tone. In this study we evaluated whether the ACE gene I/D polymorphism was a risk factor for SIH or SVD. 58 patients with SIH (mean age: 58.9 +/-11.6 years), 70 patients with ischemic stroke due to SVD (mean age: 64.4 +/- 10.6 years) and 256 healthy controls matched for age and sex, were genotyped for ACE I/D polymorphism. All patients with SIH underwent computed tomography, angiography, and where needed, MRI or MRA to exclude vascular malformations, hemorrhage to tumor, amyloid angiopathy etc. SVD ischemic stroke was diagnosed according to TOAST criteria. The ACE gene polymorphism was detected by PCR of intron 16 specific I/D fragments, 490kb, and 190kb, respectively. The ACE genotype distribution in patients with SIH (II-17.3%, ID-37.9%, DD-44.8%) differed significantly from controls (II-23.3%, ID-49.2%, DD-27.5%) (p<0.05). ACE genotype distribution in patients with SVD stroke and their controls showed no significant difference. A logistic regression model demonstrated that the DD genotype of ACE gene is independent from hypertension as a risk factor for SIH (OR=2.14, 95%CI: 1.01-4.53). Thus, the DD genotype of ACE gene is a risk factor for SIH, but not for ischemic stroke related to SVD. These two small cerebral vessel diseases differ with respect to this aspect of their genetic predispositions.

**X Chromosome Inactivation and Candidate Gene Studies in Females with Autism.** *Z. Talebizadeh, D.C. Bittel, N. Kibiryeve, M.G. Butler.* Children's Mercy Hospitals and Clinics and University of Missouri-Kansas City School of Medicine, Kansas City, MO.

Autism is a neurodevelopmental disorder with sex ratio of 3-4 times higher in males than in females. It is not clear why autism is more prevalent in males than in females. However, genes on the X chromosome may contribute to the excess number of males, which is further supported by males having only one X chromosome. As a result of X chromosome inactivation, females in the general population are mosaic for X-linked gene expression with one population of cells expressing genes from the maternal X and the other population expressing genes from the paternal X chromosome. X inactivation is generally random with an expected mean inactivation pattern of 50:50. Deviation from the 50:50 ratio (greater than 80:20) is referred to as X chromosome skewing. If a female is heterozygous for a gene predisposing to autism, X chromosome skewing may contribute to autism. We studied X chromosome skewing in females diagnosed with classical autism (N=26; 8 simplex and 18 multiplex) compared with similarly aged unaffected female siblings and/or controls with the use of the androgen receptor (AR) gene as a marker for X chromosome skewing. The age range was 5 to 12 years. The frequency of the methylated (inactive) and unmethylated (active) alleles were determined and skewness pattern characterized by methyl sensitive restriction enzyme (HhaI or HpaII) digestion followed by genotyping and measurement of the ratio of the allele peak heights. A trend for X chromosome skewing was detected (Fisher exact test,  $p=0.071$ ) in our autism group (6 out of 22) compared to unaffected females (1 out of 19). The promoter region of the XIST gene involved in X chromosome inactivation, has been analyzed for the presence of a mutation associated with X chromosome skewness in the relevant subjects but no mutations seen. Additionally, X chromosome candidate genes for autism were evaluated including gastrin-releasing peptide receptor (GRPR) and two X-linked neuroligins (NLGN3 and NLGN4) using quantitative RT-PCR and direct DNA sequencing. To date, no mutations have been found in these candidate genes in our autism subjects.

**Markers associated with the inversion polymorphism on chromosome 8p23 are associated with Panic Disorders.**

*T.E. Thorgeirsson<sup>1</sup>, H. Oskarsson<sup>2</sup>, S. Bjornsdottir<sup>1</sup>, C. Lopez-Correa<sup>1</sup>, N. Desnica<sup>1</sup>, J. Pop Kostic<sup>1</sup>, N. Vidarsdottir<sup>1</sup>, J.G. Stefansson<sup>3</sup>, H. Kolbeinsson<sup>3</sup>, E. Lindal<sup>3</sup>, J. Sainz<sup>1</sup>, N. Gagunashvili<sup>1</sup>, E. Gudfinnsson<sup>1</sup>, M.F. Frigge<sup>1</sup>, A. Kong<sup>1</sup>, J.R. Gulcher<sup>1</sup>, K. Stefansson<sup>1</sup>.* 1) deCODE genetics, Reykjavik, Iceland; 2) Therapeia, Reykjavik, Iceland; 3) Landspítalinn-University Hospital, Reykjavik, Iceland.

We report the association of the less frequent form of the inversion polymorphism on chromosome 8p23 to Panic Disorder (PD). We initially studied the chromosomes of 43 individuals with PD by fluorescence in-situ hybridization (FISH), and found 50% of the chromosomes having the rarer order, but its frequency has been estimated at 26% in European populations (Giglio et al, *Am J Hum Genet*, 68(4):874-83). We subsequently identified surrogates for the inversion status by analyzing allelic association of microsatellite markers and SNPs in the region in a larger group of individuals with known status for the inversion. Several excellent surrogates were identified, alleviating the need for further FISH. The conclusions from the FISH measurements were confirmed in a larger sample using the surrogate markers, with several markers at 8p23 exhibiting association to PD with highly significant p-values. In all cases the alleles associated to PD are the same alleles that are associated to the rarer form of the inversion. Specifically, alleles strongly associated with the inversion have a relative risk of 1.8 for PD compared to the other alleles. Weaker associations and lower risk ratios were detected for bipolar disorder and depression severe enough to require medication. Whether the inverted segment plays an active role casting PD as a genomic disorder, or serves as a background for another genetic variation increasing the risk of psychiatric disorders will be discussed.

**Evidence for the involvement of the BDNF gene in autism and relation to family history of depression.** *A.M. Vicente<sup>1</sup>, S. Silva<sup>1</sup>, A.M. Coutinho<sup>1</sup>, C. Bento<sup>2</sup>, C. Marques<sup>2</sup>, A. Atade<sup>2</sup>, T. Miguel<sup>2</sup>, L. Borges<sup>2</sup>, G. Oliveira<sup>2</sup>.* 1) Instituto Gulbenkian de Ciencia, Oeiras, Portugal; 2) Hospital Pediatrico de Coimbra, Coimbra, Portugal.

The Brain-Derived Neurotrophic Factor (BDNF) plays a fundamental role in the regulation of neural growth, differentiation and function. Abnormal neonatal levels of BDNF in children who were later diagnosed with autism have been reported, and there is growing evidence for an association of the BDNF gene with affective disorders. Particularly relevant to the etiology of autism, BDNF plays an important role in the development, function, and regeneration of serotonergic neurons. A microsatellite marker 1kb upstream of the BDNF start site and a functional SNP in exon 1 of the BDNF gene were tested for association with autism in a sample of 195 nuclear families with one affected child. The TDT for haplotypes of the two markers was significant ( $P=0.029$ ), with a distortion in the transmission of haplotype 2 ( $P=0.0071$ ). In our idiopathic sample, we find that 12% of patients have a family history of depression, a much higher rate than is found in the patients with known etiology and which therefore cannot be explained by the burden of raising a handicapped child. We further investigated the hypothesis of a common underlying genetic defect in autism and depression by restricting our analysis to the 24 patients whose parents reported having suffered episodes of depression. Comparing haplotype frequencies between patients with and without depressed parents we found haplotype 1 significantly more common ( $P=0.005$ ) and haplotype 4 significantly less common ( $P=0.003$ ) in the children of depressed parents. Because BDNF interacts with the serotonin system at multiple levels, and hyperserotonemia is a consistent feature in autism, we tested whether BDNF variants contribute to serotonin level variation in autism patients. QTDT analysis revealed no association of BDNF with serotonin distribution. These results provide evidence for an association of the BDNF gene with autism, in particular in the subgroup of autistic patients with depressed parents, and thus reinforce a role of BDNF in the etiology of autism.

Program Nr: 2023 from 2003 ASHG Annual Meeting

**Williams Syndrome: evidence from atypical deletions links GTF2I and GTF2IRD1, but not FZD9, with posterior cortical structure and neural functions.** *A.F. Simon<sup>1</sup>, G.M. Yao<sup>1</sup>, X.-N. Chen<sup>1</sup>, D. Mills<sup>2</sup>, F.E. Rose<sup>3</sup>, L.S.*

*Salandanan<sup>1</sup>, A. Reiss<sup>4</sup>, U. Bellugi<sup>3</sup>, J.R. Korenberg<sup>1</sup>.* 1) Med Genet, Cedars-Sinai Medical Center, UCLA, Los Angeles, CA; 2) Emory Univ., Atlanta, GA; 3) The Salk Institute, San Diego, CA; 4) Dept of Psychiatry and Behavioral Sciences, Stanford Univ School of Medicine, Stanford, CA.

Williams syndrome (WS) is a neurodevelopmental disorder caused by a deletion of chromosome 7q11.23. Individuals exhibit striking peaks and valleys in neurocognition that include deficits in visuospatial processing along with relatively preserved language and facial processing. These features are associated with distinct variations in neuroanatomy and physiology. In an effort to identify their genetic origins, we have used studies of molecular structure, volumetric magnetic resonance imaging (MRI), neurocognition, and event-related potentials (ERPs) in 71 individuals with WS characteristics. We used multicolor FISH, with a panel of 45 BACS, PACS and cosmids, PCR of somatic cells hybrids and quantitative southern blots to identify 69 adults with typical deletions, and 2 adult males with overlapping atypical deletions (one deleting from FKBP6 through CYLN2, and the second deleting CLDN4 through GTF2I). These individuals' breakpoints provide models with which to test hypotheses linking neural profiles with the deletion effects of 3 regions: FKBP6/FZD9-TBL2, CLDN3/4-CYLN2 and GTF2IRD1-GTF2I. Combining genetic with neural information, we propose that decreased expression of 2 transcriptional regulators, GTF2IRD1 and GTF2I, but not of FZD9, is related to a distinct subset of WS features that includes posterior cerebral variation, visuospatial processing (copy tasks and line orientation), and abnormal neural activity (ERPs) for face processing. The results of this study begin to define pathways linking gene expression with human cognition.

**A scyllatoxin-insensitive isoform of the human SK3 channel.** *O.H. Wittekindt*<sup>1, 2</sup>, *V. Visan*<sup>1</sup>, *H. Tomita*<sup>3, 4</sup>, *F. Imtiaz*<sup>3, 5</sup>, *J.J. Gargus*<sup>3, 6</sup>, *F. Lehmann-Horn*<sup>1</sup>, *S. Grissmer*<sup>1</sup>, *D.J. Morris-Rosendahl*<sup>2</sup>. 1) Department of Applied Physiology, University of Ulm, Ulm, Germany; 2) Institute of Human Genetics and Anthropology, Albert-Ludwigs-University, Freiburg, Germany; 3) Department of Physiology & Biophysics, University of California, Irvine, CA; 4) Department of Psychiatry, University of California, Irvine, CA; 5) King Faisal Medical Center, Riyadh, Saudi Arabia; 6) Division of Human Genetics, University of California, Irvine, CA.

SK ion channels mediate the after hyperpolarisation (AHP) and are shown to modulate the spike frequency of excitable cells. In the rat SK3 channels function as pacemakers in dopaminergic neurons, blockage causing bursts of action potentials and increased dopamine release, providing SK3 a candidate role in dopaminergic transmission, a process hypothesized to be affected in schizophrenia. The *KCNN3/SK3* gene is located on chromosome 1q21.3, a region implicated in schizophrenia. It contains a polymorphic CAG repeat within its first coding exon for which an association with schizophrenia has been extensively studied. We have isolated a novel SK3 isoform from human embryonic cDNA, SK3\_ex4, that contains a 15 amino acid insertion within the S5 to P loop segment. Alternative splicing has been recognized to be important in increasing the functional diversity of ion channels. Transcripts encoding SK3\_ex4 are co-expressed with SK3 but at lower levels in neuronal as well as in non neuronal tissues. In order to investigate the pharmacokinetic properties of SK3\_ex4, we expressed the isoforms SK3 and SK3\_ex4 in tsA cells. The most obvious distinguishing feature of this new isoform was that while SK3 was blocked by scyllatoxin (K<sub>d</sub> = 2.1 nM) and d tubocurarine (K<sub>d</sub> = 5.4 M), hSK3\_ex4 was not affected by either. Both isoforms were similarly activated by cytosolic Ca<sup>2+</sup> and by 1 ethyl 2-benzimidazolinone (1 EBIO), blocked by TEA<sup>+</sup> and Ba<sup>2+</sup>, and showed similar permeabilities relative to K<sup>+</sup> for Cs<sup>+</sup> and Rb<sup>+</sup>. These features are compatible with SK3\_ex4 underlying the recognized toxin-insensitive component of the afterhyperpolarisation in excitable cells and perhaps in disorders of dopamine release.

**Mapping the genetic architecture of Tau H1 in neurodegeneration.** *M. Toft*<sup>1,2</sup>, *L. Skipper*<sup>1</sup>, *K. Wilkes*<sup>1</sup>, *S. Lincoln*<sup>1</sup>, *J. Aasly*<sup>2</sup>, *M. Farrer*<sup>1</sup>. 1) Neuroscience, Mayo Clinic Jacksonville, Jacksonville; 2) Department of Neurology, St. Olav's Hospital, Trondheim, Norway.

The tau H1 haplotype has been associated with 4R tauopathies including progressive supranuclear palsy, corticobasal degeneration and argyrophilic grain disease. More controversially the same haplotype has been associated with Parkinson's disease. Using H1-specific SNPs we demonstrate tau H1 is a misnomer, which consists of a family of recombining H1 alleles. Population genetics, linkage disequilibrium and association analyses have shown that one discrete haplotype is preferentially associated with parkinsonism. Using a sliding scale of H1-specific haplotypes, in age/gender matched Parkinson's disease cases and controls from central Norway, we have refined the association within individuals with global tau H1/H1 genotypes to a ~40kb interval within the tau gene. This haplotype is highly associated with disease ( $p < 2e-6$ ). Hypothesis to explain the enigmatic association of both tauopathy and synucleinopathy with tau H1 are explored. An update on the genetic, genomic and functional analysis of marker variability within this region is presented.

**Identification of ethyl mercury-related genetic determinants that may increase susceptibility towards autism using a yeast deletion based assay.** . A.B. Shah<sup>1</sup>, J. Faustino<sup>1</sup>, R. Zordan<sup>2</sup>, S. Angeli<sup>2</sup>, J. Gitschier<sup>1,2</sup>. 1) Howard Hughes Medical Institute; 2) Departments of Medicine and Pediatrics, University of California at San Francisco, San Francisco, CA.

Thimerosal, a preservative composed of 49.6% ethyl mercury, has been used in several pediatric vaccines distributed around the world. Several studies, illustrating the toxic effects of other forms of mercury on the brain and nervous system, led to the removal in 1999 of thimerosal from vaccines in the United States; however, multidose vials of vaccines containing thimerosal are still being used for childhood immunization in developing countries. This preservative has become a major source of mercury in children who, within their first two years of life, may have received a quantity of mercury exceeding safety guidelines.

The similarities between the characteristic traits defining or associated with autism and the sensory, neurological, motor, and behavioral dysfunctions resulting from toxic mercury exposure suggest that some cases of regressive autism may be induced by early mercury exposure from thimerosal. To investigate the plausibility for a connection between thimerosal and autistic spectrum disorders, we have carried out a large scale screen using a haploid *Saccharomyces cerevisiae* yeast deletion library to identify strains carrying a single null allele exhibiting ethyl mercurial ion sensitivity. We have identified a number of genes involved in several diverse cellular processes, including signal transduction, vesicular trafficking, microtubule formation, proline biosynthesis, chromatin silencing, RNA transcription, and the oxidative stress response. Furthermore, we have discovered genetic players with unknown function, some of which may have a direct role in mercurial export since thus far, no eukaryotic genes involved in mercury homeostasis have been identified. We propose that genetic alterations affecting mRNA levels, gene function, or gene dosage of specific genes may establish a predisposition whereby thimerosal's adverse effects occur only in some children.

**Identification of *COL24A1* as a novel candidate gene for heart failure in mice and humans.** *K.M. Carlson, E.R. Hauser, M.P. Donahue, W.E. Kraus, H.A. Rockman, D.A. Marchuk.* Duke University Medical Center, Durham, NC.

Heart failure (HF) is a complex disease, exhibiting phenotypic variability in outcome related to both environmental and genetic factors. Although biological candidate genes have been investigated as risk factors, identification of novel loci has been largely unsuccessful. We have used a mouse model of HF as a means to identify genetic risk factors. The HF phenotype of a transgenic mouse with cardiac-specific over-expression of the Ca<sup>++</sup> binding protein, calsequestrin (CSQ), shows strong genetic background (strain-specific) effects. We previously described the use of this model to identify a major modifier locus, *Hfm2*, on mouse chromosome 3, affecting both heart function and survival. We now report further analysis of this locus. We screened 412 N2 backcrossed (F1(CSQ)XDBA) progeny for crossovers in the *Hfm2* interval and determined survival for all animals with a crossover within this region. Three proximal and two distal crossovers define a new 1.1 Mb interval, with only two discordant animals. This interval contains 12 annotated genes, 4 of which are not known to be expressed in the heart. DNA sequencing of the remaining 8 genes in DBA and C57Bl/6 strains identified coding polymorphisms (3 SNPs) in only one novel gene. Through the use of mouse/human synteny, we identified the novel gene as collagen type XXIV, alpha 1. We also identified 30 SNPs in the putative promoter region of the gene. RT-PCR analysis indicates that the transcript is up-regulated in this model of HF. These data, as well as the role of collagens in the remodeling that occurs during HF, make *COL24A1* a novel candidate gene for human HF. We sequenced *COL24A1* in 16 human patients with HF, and identified 11 nonsynonymous cSNPs. These were typed in a panel of 243 HF patients. Preliminary data shows a potential association ( $p < 0.06$ ) between V61A(182t->c) and disease severity, as measured by ejection fraction. We are currently collecting additional HF patients and identifying additional SNPs in the gene for further analysis.

**Novel COL6A1 variants and congenital heart defects (CHD) in Down Syndrome (DS).** *U. Fairbrother<sup>1</sup>, M.J. Baptista<sup>2</sup>, G.E. Davies<sup>1</sup>, D. Triikka<sup>1</sup>, A.M. Kessling<sup>1</sup>.* 1) Med & Community Genetics, Imperial College, London, United Kingdom; 2) Laboratory of Neurogenetics, Bethesda, MD, USA.

Free trisomy 21 pathology is unlikely to be due simply to over expression of chromosome 21 genes, since only 40% of individuals with DS have a CHD. Much evidence suggests phenotypic variation is related to specific alleles, and protein products functioning suboptimally in trisomic cells. We identified the COL6A1-A2 gene cluster as a CHD critical region. In individuals with partial trisomy and DS + CHD, COL6A1 has never been excluded from a causal role. We screened 91% of the COL6A1 coding region in 97 families, including individuals with DS +/- CHDs. Of those 19 had complete AV canal defect (AVSD), 11, ventricular septal defect (VSD) and 19 a combination/other CHD. There is a higher than expected level of coding and non-coding variation in COL6A1, with over 30 variable sites identified including mutations in the collagen VI 1 chain C-terminal globular region. We identified novel amino acid changes in the triple-helical (TH) region that may perturb heterotrimer formation with 2 and 3 chains, in native collagen VI monomers. COL6A1 genomic analysis identifies a putative cryptic splice site that may result in alternative splicing and detects an intron conserved more highly than the flanking exons. The next intron has exceptional length and sequence variation suggestive of a recombination hot spot: there may be a similarly polymorphic region in the analogous COL6A2 position. We have evidence that a subset of these haplotypes discriminates between individuals with DS or DS + CHD. Collagen VI tertiary structure may affect complex higher order interactions during heart development. Recent published evidence shows that trisomic 21 fibroblasts have increased adhesive capacity for extracellular collagen VI through 1 integrin interaction. We propose that alterations in higher order structure of collagen VI protein result from combinations of mutations embedded in particular COL6A1 haplotypes. This may lead to altered trisomic cell adhesiveness resulting in a single or several types of CHD.

**An Investigation of the Matrix Metalloproteinase-3 -1612 5A/6A Polymorphism in Ischemic Heart Disease using Family Based Tests of Association.** *P.G. McGlinchey<sup>1</sup>, M.S. Spence<sup>1</sup>, G. Murphy<sup>1</sup>, A.R. Allen<sup>2</sup>, C. Patterson<sup>3</sup>, P.P. McKeown<sup>2</sup>.* 1) Department of Cardiology, Royal Victoria Hospital, Belfast, United Kingdom; 2) Department of Medicine, Queen's University of Belfast, United Kingdom; 3) Department of Epidemiology & Public Health, Queen's University of Belfast, United Kingdom.

**Introduction.** Atherosclerosis is the pathological basis of ischemic heart disease (IHD). Matrix metalloproteinases (MMPs) are enzymes important in vascular remodelling, a feature of atherosclerosis. Increased MMP activity predisposes to atherosclerotic plaque rupture, a feature of acute myocardial infarction and unstable angina. In contrast, reduced MMP activity may cause decreased matrix degradation, leading to increased plaque size. MMP3 has been found in plaques and has the broadest spectrum of activity of all MMPs. A common polymorphism in the promoter region of the MMP3 gene, the -1612 5A/6A polymorphism, has functional importance in vitro, with the 5A allele associated with increased MMP3 expression. Case-control studies have shown conflicting findings of the role of this polymorphism in IHD. We investigated this polymorphism in a well-defined IHD population using two family based tests of association. **Methods.** Families with at least one individual with premature onset IHD (males 55 years, females 60 years) with all grandparents born in Ireland were recruited. Genotyping was performed using a PCR-RFLP method. Analysis was performed using the combined transmission disequilibrium test (TDT)/sib-TDT and the pedigree disequilibrium test (PDT). These tests are unaffected by population admixture. **Results.** 388 families consisting of 1023 individuals (418 affected cases, 110 parents, and 495 unaffected siblings older than the proband was at IHD onset) were recruited and genotyped. The -1612 5A/6A polymorphism was demonstrated to be not associated with IHD by either the combined TDT/sib-TDT ( $p=0.16$ ) or the PDT ( $p=0.06$ ). The study afforded over 80% power to detect a deviation of allele transmission from 50 to 60% ( $p0.05$ ). **Conclusion.** The MMP3 -1612 5A/6A polymorphism is not associated with premature-onset IHD in the Irish population.

**Atrioventricular septal defects in *Creld1*-deficient mice reflect the association of *CRELD1* mutations with heart defects in humans.** *C.L. Maslen, G.T. Fouad, S.W. Robinson.* Dept Molec/Med Genetics, Oregon Health & Science Univ, Portland, OR.

Atrioventricular septal defects (AVSD) are commonly occurring heart malformations, and are the most frequently diagnosed birth defect in the first year of life. The atrioventricular valves and septa form from progenitor cardiac structures called endocardial cushions. AVSD represents a failure of endocardial cushion development, resulting in incomplete septation. *CRELD1* is a novel membrane bound glycoprotein with characteristics of a cell adhesion molecule. The *CRELD1* gene maps to chromosome 3p25.3 in the vicinity of the *AVSD2* locus. Missense mutations in *CRELD1* are associated with AVSD in humans (Robinson, AJHG, 2003). In that study we found that about 6% of individuals with non-Down syndrome related AVSD have a *CRELD1* mutation. There is incomplete penetrance, indicating that *CRELD1* is a genetic risk factor for AVSD. To evaluate the specific role of *CRELD1* in heart development, we created a mouse model that has been genetically altered to eliminate the *Creld1* gene. Examination of *Creld1*<sup>-/-</sup> embryos shows that they die *in utero* at approximately embryonic day E11.0. Histopathologic analysis indicates that *Creld1*<sup>-/-</sup> mice have multiple heart malformations. The atrioventricular endocardial cushions are underdeveloped and have a reduced number of mesenchymal cells migrating into the cushions, as is required for normal development. Mice heterozygous for the disrupted allele (*Creld1*<sup>+/-</sup>) are usually viable and fertile with no obvious signs of abnormalities. However, a small subset of *Creld1*<sup>+/-</sup> embryos has pericardial edema. Those embryos have a deficiency in cellularization of the atrioventricular endocardial cushions similar to that seen in the null embryos, but the heart defects appear to be less severe than those seen in *Creld1*<sup>-/-</sup> embryos. These data demonstrate that *CRELD1* function is required for atrioventricular septal morphogenesis. Haploinsufficiency results in endocardial cushion defects with incomplete penetrance. The failure of endocardial cushion cellularization in *Creld1*-deficient mice is consistent with the association between *CRELD1* mutations and AVSD in humans.

**Involvement of chromosome Xp11.22 region in the human hypertension.** *M.Z. Labuda<sup>1</sup>, J. Uwabo<sup>1</sup>, Y. Sun<sup>1</sup>, D. Gaudet<sup>2</sup>, F. Gossard<sup>1</sup>, T. Kotchen<sup>3</sup>, A.W. Cowley<sup>3</sup>, J. Tremblay<sup>1</sup>, P. Hamet<sup>1</sup>.* 1) Medicine, Res.Center of Hotel Dieu Hosp , Montreal, Quebec, Canada; 2) Complexe Hospitalier de la Sagamie, Chicoutimi-Canada; 3) Medical College of Wisconsin, Milwaukee-USA.

Heat shock proteins (Hsp) play a critical role in maintaining cellular homeostasis as a protective response to different kind of stress. Hsp27 is one of such proteins involved in maintaining structural integrity and contractile function of the cardiac cell. Hsp27lp on human chromosome Xp has high similarity to Hsp27 but its functional status remains to be established. A 3UTR mRNA polymorphism resides in similar position in rat Hsp27 shown to be associated with hypertension. Here we investigated influence of this gene on hypertension status among subjects from French Canadian families from Saguenay-Lac St-Jean in Quebec. Our sample consisted of 774 individuals from 115 families (>250 sibships) selected for the presence of at least two sibs with early onset HT (55y). The analysis was carried out on 359 males and 415 females (66% and 60% hypertensive) and was performed according to gender, age and HT status. Subjects were stratified into the following groups NT<55y, NT>60y, NT 55-59y, HT with the onset 41-55y, HT with the onset >55y, HT with the onset <40y. In males, the frequency of genotype 1 (17.2%, 62/359 overall), increased with age among hypertensive and consequently decreased among NT with age. At 55-59y there was only 6.5% NT (2/31) genotype 1 male carriers, while over 60y none of 20 NT males was a carrier of this genotype. Among females divided into six groups the distribution of genotypes was significantly different ( $p=0.003$ ), in contrast to males, with overrepresentation of genotype 2/2 among HT. In a group by group comparison no significant different was observed but genotype 2 appeared to be a risk for developing early HT. Among females with late HT onset (55y) the frequency of genotype 1/1+1/2 increased to 41% comparing to 16% in whole female sample suggesting an influence of genotype 1 on HT among postmenopausal women. In conclusion: Our data suggests involvement of chromosome Xp11.22 region (possibly Hsp27lp) in human hypertension with a specific impact of age in each gender.

**QTLs for Atherosclerotic Lesions and Aortic Aneurysms in the C57BL/6J and C3H/HeJ Mouse Models. S.**

*Wang<sup>1</sup>, W.-B. Shi<sup>2</sup>, X.-P. Wang<sup>1</sup>, A. Ghazalpour<sup>1</sup>, A.J. Lusis<sup>1</sup>.* 1) Human Genetics, University of California, Los Angeles, CA; 2) Radiology, University of Virginia, Charlottesville, VA.

Inbred mouse strains C57BL/6J and C3H/HeJ differ strikingly in susceptibility to atherosclerosis on an apoE<sup>-/-</sup> background. B6.apoE<sup>-/-</sup> mice develop lesions readily whereas C3H on the same background are resistant. In order to identify the genes responsible for the difference in susceptibility, B6.apoE<sup>-/-</sup> and C3H.apoE<sup>-/-</sup> mice were crossed to generate 135 female F2 progeny for QTL analysis. The mice were fed on chow for 10 weeks, sacrificed, and a genome scan was conducted. The F2s were phenotyped for aortic lesions, calcification, aneurysms, weight, total cholesterol, HDL, LDL, free fatty acids, and glucose levels. QTLs for aortic lesions were found on proximal chromosome 9 (D9mit25) with a LOD score of 6.2, and on proximal chromosome 4 (D4mit111) with a LOD score of 3.0. A QTL for aneurysms was found over the same region of chromosome 4 with a LOD score of 4.8. We suspect the chromosome 4 QTL is caused by Toll-Like Receptor 4, an LPS receptor that is known to be nonfunctional in C3H mice due to a missense mutation. The chromosome 9 QTL is being further investigated through the construction of congenic mice. We hypothesize that genes involved in inflammatory responses are the primary determinants of atherosclerosis susceptibility in this model, as it has been shown in vitro that aortic endothelial cells isolated from B6 exhibit inflammatory response when treated with minimally oxidized LDL, while C3H endothelial cells fail to be induced.

**Genetics of salt sensitive and low renin hypertension.** *S.M. Williams<sup>1</sup>, J. Yatabe<sup>2</sup>, S. Midorikawa<sup>2</sup>, S. Hashimoto<sup>2</sup>, T. Watanabe<sup>2</sup>, J.H. Moore<sup>1</sup>, M.D. Ritchie<sup>1</sup>, P.A. Jose<sup>3</sup>, R.A. Felder<sup>4</sup>, H. Sanada<sup>2</sup>.* 1) Prog Hum Genet, Vanderbilt Univ Med Ctr, Nashville, TN; 2) Fukushima Med Univ, Fukushima, Japan; 3) Georgetown Univ Med Ctr, Washington, DC; 4) Univ of VA Health Sciences Ctr, Charlottesville, VA.

Salt sensitive hypertension is a genetic condition that affects millions of people world-wide. However, clinical diagnosis of salt sensitivity (SS) is difficult. Therefore, low renin (LR) hypertension is usually used as a proxy. We tested the hypothesis that these two conditions have the same underlying genetic basis by determining the best genetic model for each and comparing them. We studied 83 Japanese subjects (35 SS and 48 non-SS hypertensives) for SS and 131 Japanese subjects for renin status (70 LR and 61 normal/high renin hypertensives). For each cohort we studied 12 polymorphisms in 9 loci (3 SNPs in the G protein-coupled receptor kinase type 4 - GRK4; 2 SNPs in angiotensinogen, the indel variant in angiotensin converting enzyme, and 1 SNP in each of the following genes - AT1 receptor, CYP11B2, D1 receptor, alpha-adducin and G-protein beta3 subunit). We analyzed single site associations for the phenotypes and found significant associations for both SS and LR hypertension for all three SNPs in the GRK4 gene, and for LR hypertension for the CYP11B2 and alpha-adducin genes. In addition to single site analysis, we used multifactor dimensionality reduction (MDR) to determine the best genetic model for each phenotype. In the case of SS hypertension a 3 locus model incorporating all three GRK4 variants successfully predicted the phenotype correct 94.4% of the time. However, despite the single locus effects found for LR hypertension with all 3 variants in the GRK4 and the CYP11B2 and alpha-adducin genes, the best MDR model incorporated only one of the GRK4 variants and the SNP in the CYP11B2 (Prediction 84.5%). These results illustrate two important points: 1) the genetic basis of SS and LR hypertension, although having some overlap are not identical, suggesting that these two phenotypes are not the same and 2) interactions among genes may be more important in the genetics of complex phenotypes than marginal effects..

**Association of APOC3 -455T/C genotypes with coronary artery disease risk in Metabolic Syndrome patients.** *E. Trabetti<sup>1</sup>, O. Olivieri<sup>2</sup>, F. Pizzolo<sup>2</sup>, N. Martinelli<sup>2</sup>, D. Girelli<sup>2</sup>, A. Bassi<sup>3</sup>, R. Corrocher<sup>2</sup>, P.F. Pignatti<sup>1</sup>.* 1) Mother-Child & Biol-Genetics, Univ. Verona, Verona, Italy; 2) Dept. Clinical & Experimental Medicine, Univ. Verona, Verona, Italy; 3) Institute of Clinical Chemistry, Univ. Verona, Verona, Italy.

**Background.** We have recently demonstrated (1) that homozygosity for APOC3 -455T/C variant represents an independent susceptibility factor for coronary artery disease (CAD). This variant has a reduced affinity for the nuclear factors mediating the inhibitory insulin effect on the apo CIII gene transcription, so that it is considered the first example of insulin resistance at the gene level. Metabolic Syndrome includes obesity, hypertension, diabetes or glucose intolerance and disorders of lipid metabolism, and it is linked to insulin activity. **Materials and Methods.** We studied a total of 873 patients, 549 of whom had angiographically documented coronary atherosclerosis, whereas 251 had normal coronary arteriograms. Patients were also classified as having the metabolic syndrome (MS, n=270) or not (MS-free, n=603) according the Adult Treatment Panel III (ATP III) criteria. We measured plasma lipids, insulin and apolipoproteins A1, B, E, C-III and assessed APOC3 -455T/C genotype of all patients. **Results.** In CAD patients, 17.7% were homozygous for the -455C variant compared to 10.2% in CAD-free group ( $p < 0.02$ ), whereas there was no difference in genotype frequency between MS and MS-free group (15.2 vs 15.4, NS). However, OR for CAD in MS patients was increased in individuals carrying -455C: OR=5.988 (2.88-12.42) in heterozygotes, and OR=9.276 (2.17-39.6) in CC homozygotes. The -455C variant was also associated with increased apo CIII and TG levels. **Conclusions.** APOC3 -455T/C genotypes contribute to increase the CAD risk associated with the Metabolic Syndrome, and are associated with increased plasma levels of TG-rich lipoproteins and apo CIII. (1) O. Olivieri et al. Apo CIII gene polymorphisms and risk of coronary artery disease. *Journal of Lipid Research*, 43:1450-1457, 2002.

**PON2 Ser311Cys genotype, and its interaction with smoking, increase the risk of myocardial infarction in patients with coronary atherosclerosis.** *P.F. Pignatti<sup>1</sup>, D. Girelli<sup>2</sup>, N. Martinelli<sup>2</sup>, O. Olivieri<sup>2</sup>, F. Pizzolo<sup>2</sup>, S. Friso<sup>2</sup>, I. Tenuti<sup>2</sup>, R. Corrocher<sup>2</sup>, M. Grow<sup>3</sup>, S. Cheng<sup>3</sup>, E. Trabetti<sup>1</sup>.* 1) Dept Mother & Child/Biol & Gen, Univ Verona, Verona, Italy; 2) Dept. Clinical & Experimental Medicine, Univ. Verona, Verona, Italy; 3) Dpt. Hum. Genetics, Roche Molecular Systems, Inc., Alameda, CA, USA.

**Background.** Increased oxidative stress is a major determinant of endothelial dysfunction, involved in the pathogenesis of the atherothrombotic process. Paraoxonases (PON) are a group of enzymes with antioxidant properties. Three common polymorphisms have been described in PON genes (PON1 Leu55Met and Gln192Arg; PON2 Ser311Cys), with possible implications for the risk of coronary atherosclerotic disease (CAD) or its main thrombotic complication, myocardial infarction (MI). **Methods.** 890 subjects were subjected to angiographic documentation of coronary vessels (272=CAD-free; 618=CAD). In the CAD group, 341 subjects had documentation of MI. All the conventional risk factors for CAD and the above mentioned PON1 and PON2 gene polymorphisms were assessed. The interaction of the polymorphisms with smoking, the main environmental factor involved in oxidative damage, was evaluated. **Results.** An increased frequency of heterozygotes and homozygotes for PON2 311Cys were present among CAD subjects with MI than among CAD subjects without MI (35.5% vs 28.8%, and 7% vs 3.6%, respectively; P 0.01). By multivariate logistic regression analysis, the adjusted OR for MI among PON2 311Cys carriers was 1.5 (95% CI, 1.03-2.19). Smoking was associated to an increased risk of MI (OR=1.6; 95% CI, 1.2-2.3). The risk of MI in smokers was related to PON2 Ser311Cys genotypes, being significantly increased in Cys/Cys homozygotes (OR=5.6; 95% CI, 1.7-16.4) and heterozygotes (OR=2.1; 95% CI, 1.3-3.6), but not in Ser/Ser homozygotes (OR=1.2; 95% CI, 0.8-1.8). PON2 Ser311Cys genotype did not influence risk of MI in non-smoker CAD. **Conclusions.** In subjects with CAD, MI risk may be influenced by the interaction between PON2 Ser311Cys genotype and smoking.

**Adrenergic receptor polymorphisms associated with resting heart rate: The HyperGEN Study.** *J.B. Wilk<sup>1</sup>, R.H. Myers<sup>1</sup>, J. Pankow<sup>2</sup>, S.C. Hunt<sup>3</sup>, M.F. Leppert<sup>3</sup>, R.C. Ellison<sup>1</sup>.* 1) Boston University School of Medicine, Boston, MA; 2) University of Minnesota School of Public Health, Minneapolis, MN; 3) University of Utah, Salt Lake City, UT.

Polymorphisms in the adrenergic receptor (AR) genes have been reported to be associated with cardiovascular function, including 1 AR polymorphisms associated with resting heart rate. We evaluated the association of polymorphisms in the 1, 2 and 2B ARs with resting heart rate in groups of participants in the HyperGEN Study: hypertensive and normotensive whites and African-Americans. Because beta-blockers are known to influence heart rate, hypertensives using these medications were evaluated separately. All analyses were adjusted for age, sex, body mass index, alcohol use, smoking status, and measures of daily exercise. The Ser49Gly polymorphism of the 1 AR was significantly associated with resting heart rate in two groups. In hypertensive African-Americans not taking beta-blockers, carriers of the Gly allele had a higher mean resting heart rate by 4.7 beats per minute compared to Ser allele homozygotes ( $p=0.008$ ). In contrast, the Gly homozygotes in the normotensive white sample had a mean resting heart rate 4.1 beats per minute lower than the Ser allele carriers ( $p=0.007$ ). The 2 AR polymorphism Gly16Arg was associated with lower mean heart rate in the sample of white hypertensives taking beta-blockers ( $n=103$ ), where the Arg homozygotes had 5.8 beats per minute lower mean heart rate ( $p=0.03$ ). The 2B C/A polymorphism was associated with heart rate in the small sample ( $n=33$ ) of hypertensive African-Americans taking beta-blockers, where carriers of the A allele had higher mean resting heart rate by 7.8 beats per minute compared to C homozygotes ( $p=0.03$ ). In summary, the Ser49Gly polymorphism of the 1 AR was associated with heart rate, but opposite effects were seen for different racial and hypertension status groups. The increased sympathetic nervous system activation that occurs with hypertension may modify the effect of the Ser49Gly polymorphism on heart rate. Polymorphisms in the 2 and 2B ARs exhibit evidence for association with heart rate among hypertensives treated with beta-blockers.

**Genome-wide scan for conotruncal heart defect loci in a canine model.** *P. Werner<sup>1</sup>, M.G. Raducha<sup>1</sup>, U. Prociuk<sup>1</sup>, E.O. Ostrander<sup>2</sup>, R.S. Spielman<sup>3</sup>, E.F. Kirkness<sup>4</sup>, D.F. Patterson<sup>1</sup>, P.S. Henthorn<sup>1</sup>.* 1) School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; 2) Clinical Research and Human Biology Divisions, Fred Hutchinson Cancer Research Center, Seattle, WA; 3) School of Medicine, University of Pennsylvania, Philadelphia, PA; 4) The Institute for Genomic Research, Rockville, MD.

Isolated (non-syndromic) congenital heart defects (CHDs) are among the most common birth defects in humans and dogs. In both species, inheritance patterns are usually non-Mendelian and multifactorial inheritance has been assumed. Conotruncal defects (CTDs), a subgroup of CHDs, consist of a spectrum of defects of the conus septum, conal ventricular septal defects, tetralogy of fallot, and persistent truncus arteriosus. We performed a genome-wide scan for CTD linked loci in a keeshond dog F1 backcross pedigree in which 46 of 101 offspring had CTDs. Two-point linkage analysis under a model of autosomal recessive inheritance identified regions of suggestive linkage on three canine chromosomes: CFA2, CFA9, and CFA15. None of these loci by itself accounted for segregation of CTD in the pedigree. Multipoint parametric analysis with GENEHUNTER resulted in a significant LOD score for CFA9 (corrected LOD score of 3.31) and supported linkage to loci on CFA2 and CFA15 (corrected LOD scores of 2.71 and 3.03). GENEHUNTER TWOLOCUS analysis supported the hypothesis that CTD in the keeshond is oligogenic and suggested that, in a given individual, CTD-predisposing alleles for at least two of the three loci are necessary to produce CTD. Two of the CTD-linked canine chromosome regions contain genes that when knocked out in mice produce cardiac malformations similar to keeshond CTD: CX45 on CFA9 and EDNRA on CFA15. The CTD-linked regions in the dog are orthologous to portions of human chromosomes 5, 17, and 4. Genes known to be involved in congenital heart disease or heart development excluded by these studies include HAND1, CSX, TBX5, HAND2, and CSPG2 (Versican).

**The genetics of bleeding after cardiac surgery.** *I.J. Welsby, M. Podgoreanu, J. Mathew, B. Phillips-Bute, M.F. Newman, D.A. Schwinn, M. Stafford-Smith.* Anesthesiology, Duke University Medical Center, Durham, NC.

**Introduction** While some individual prothrombotic polymorphisms have been independently associated with bleeding after cardiac surgery, the joint effects of multiple genetic variant have never been studied. Therefore, we examined the association of 19 prospectively defined candidate prothrombotic polymorphisms with bleeding after coronary artery bypass grafting (CABG) surgery. **Methods** Following informed consent, 890 adult patients undergoing primary elective CABG surgery were enrolled in the study. Renal disease, liver disease and preoperative coagulopathy were exclusion criteria. After confirming Hardy-Weinberg equilibrium, univariate and multivariate analyses were used to identify possible associations between polymorphisms and 12 hour chest tube drainage. Those with a promising univariate association were included in a final multivariate linear regression model, which also examined potential interactions between polymorphisms. Bootstrap analysis was performed to test the validity of the final model; results are expressed as the number of times the association was significant in 100 resamplings. **Results** Upon preliminary analysis, four polymorphisms appear associated with chest tube drainage. The relationships are complex and involve paired 2-way interactive effects. Final results of our analysis will be presented at the meeting. **Discussion** Strengths of our study include that it examines multiple genetic polymorphisms simultaneously, involves a large number of patients, and measures a clinically important outcome. Our study design enables identification of 2-way interactive effects of common polymorphisms, whereas studies focused on a single polymorphism may overlook such interactions. Our findings in this relatively unstudies patient population may prove very clinically important.

Program Nr: 2039 from 2003 ASHG Annual Meeting

**Effect of an *NCCT* mutation on blood pressure in the Amish.** *S. Rutherford, J.O. Green, B.D. Mitchell, A.R. Shuldiner.* Dept Medicine, Univ Maryland, Baltimore, Baltimore, MD.

Hypertension (HT) affecting approximately 20-25% of Caucasian adults is a common multifactorial disorder and a well-known predisposing risk factor for stroke, renal failure and cardiovascular disease. The condition has a genetic basis, although at present the number of genes are unknown. The molecular basis for the Mendelian forms of HT such as glucocorticoid-remediable aldosteronism, Liddles syndrome and the syndrome of apparent mineralocorticoid excess are shown to arise from mutations that increase renal salt. Previous studies have shown mutations in the thiazide-sensitive sodium-chloride cotransporter gene (*NCCT*) reduce renal salt reabsorption and blood pressure in a large Amish kindred with Gitelmans syndrome [Cruz et al., (2001) *Hypertens* 37:1458-1464]. One of the mutations identified in *NCCT* is a single base substitution (CGC->GGC) at codon 642, which substitutes a glycine for the normal arginine located in a conserved region in the cytoplasmic C-terminus of the encoded protein. This mutation results in a loss of *NCCT* function. We therefore tested this mutation in an Old Order Amish population from Lancaster County, Pennsylvania that previously shows evidence for a genetic susceptibility to blood pressure variation [Hsueh et al., (2000) *Circulation* 101:2810-2816]. For this study, we used a PCR assay to genotype the single base substitution in approximately 1400 Amish individuals. Preliminary data from association analysis showed no significant differences between 90 hypertensive individuals who either had blood pressures greater than 140/90mmHg or were currently using antihypertensive medication, and 88 normotensive individuals over 45 years of age with blood pressures less than 120/80 mmHg. We are continuing to genotype more Amish individuals and will present the results from association analysis in the expanded set of Amish individuals.

**Myocardial Ion Channel IKr-alpha-Subunit Genes KCNH2 and KCNE2: Haplotype structure and modulation of the QTc Interval.** A.S. Pfeufer<sup>1</sup>, S. Perz<sup>3</sup>, S. Jalilzadeh<sup>1</sup>, T. Illig<sup>2</sup>, J. Muller<sup>1</sup>, H. Lowel<sup>2</sup>, G. Steinbeck<sup>4</sup>, H.E.

Wichmann<sup>2</sup>, S. Kaab<sup>4</sup>, T. Meitinger<sup>1</sup>. 1) Institute of Human Genetics, TU Munich and GSF Research Center, Munich, Germany; 2) Institute of Epidemiology, TU Munich and GSF Research Center, Munich, Germany; 3) Institute of Medical Informatics, TU Munich and GSF Research Center, Munich, Germany; 4) Medizinische Klinik und Poliklinik I, Klinikum der LMU Munich, Grosshadern, Munich, Germany.

The QT interval in the surface ECG is a sensitive and specific indicator of myocardial repolarization. QT prolongation indicates repolarization disturbances leading to TdP tachycardia and sudden cardiac death. Family and sib-pair studies have identified a 36% to 52% genetic component of QT. We investigated minor gene variants of the myocardial Ion Channel IKr-alpha-Subunit and long QT syndrome disease Genes KCNH2 and KCNE2 for their influence on QT. Surface-ECG recordings from participants of a population based epidemiological survey in the Augsburg region (n=4149) were used. QT correction parameters for heart rate (RR interval, +0.153 ms/ms) age (+0.245 ms/y) and sex (+7,70 ms for female) were determined by linear regression. The combined influence of these three independent variables on QT accounted for 1,59% of its total variance by multivariate regression analysis. For genetic analysis patients on cardiac medication or those showing signs of any disease pathology in their ECGs were excluded (remaining n=1019). Measured QT values were transformed to heart rate-, age- and sex- independent QTc values. Mean QTc was 409 ms (SD +/- 15 ms) when standardized to a 45y old male with a heart rate of 60/min. Genotyping of 45 SNPs revealed strong LD within both genes. Haplotype analysis revealed the 5 major haplotypes (AF>0,05) in KCNH2 and in 2 in KCNE2. Within the KCNH2 gene several consecutive SNPs showed a significant association to QTc. Most significantly associated was KCNH2 I489I (rs740952). In GG carriers QTc was 408,1 +/- 15,4 ms (n=634), in GA heterozygotes it was 410,0 +/- 14,9 ms (n=304) and in AA homozygotes 414,6 +/- 17,3 ms (n=43) (p = 0,004). The effect of the I489I SNP was of the same quality in both males and females but found stronger in males.

**Pank4, A novel pantothenate kinase gene is a Candidate Gene for Type II Diabetes Mellitus.** *Y. Li<sup>1</sup>, L. Liu<sup>1</sup>, J. Zhao<sup>2</sup>, J. Zuo<sup>1</sup>, F. Fang<sup>1</sup>.* 1) National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Peking Union Medical College, Chinese Academy of Medical Sciences, 5# Dong Dan San Tiao, Beijing, P. R. China 100005; 2) Human Genetics Center, The University of Texas, Health Science Center at Houston, Houston TX 77030, USA.

**Purpose:** To identify the function of a SD rat novel pantothenate kinase gene(pank4).

**Methods:**Pank4 was cloned from SD rat by messenger RNA differential display and its expression has been localized in many tissues by immunohistochemistry. Several proteins concern with regulation of blood glucose have been found to interact with Pank4 protein by yeast two-hybrid. The result has been further analyzed by far-western blotting and immunoprecipitation..

**Result:**A novel pantothenate kinase gene,Pank4, which was cloned from SD rat, is related to blood-glucose regulation and had ever been named Fang-1 (GenBank Accession NO.AF399873), and shared high similarity with its human homologue (GenBank Accession No. AK001644), which was identified as the pantothenate kinase 4 (pank4) gene. Firstly, the pank4 antibody was obtained by immunizing rabbit and purified, then immunohistochemicalized to rat muscle, heart muscle, lung, liver, kidney ,pancreas, hypothyroid, brain and small intestine tissues, the pank4 protein mainly express in some kind of cells which need more energy. So we can concluded that the pank4 may be related to energy producing. Secondly, by yeast two-hybrid using the pank4 as bait, we found that pank4 was interacted with 3 beta-hydroxysteroid dehydrogenase isomerase type II, glyceraldehyde- 3-phosphate dehydrogenase, *Rattus norvegicus* pyruvate kinase, *Rattus norvegicus* glycogen Phosphorylase, by in-vivo and in vitro identification, pank4 was interacted with these proteins. The result suggested that pank4 is not only a key regulatory enzyme in the CoA biosynthesis, but also is a key regulatory enzyme in the saccharometabolism. It may play a very important role in the glucoregulation. So we proposed the the pank4 may be a novel candidate gene for type II diabetes mellitus.

**CAG repeat alteration and parental origin of the androgen receptor in Turner syndrome.** *S.M. Zeng, J. Yankowitz.*  
Dept OB/GYN, University of Iowa Roy J. and Lucille A. Carver College of Medicine, Iowa City, IA.

Turner syndrome (TS) is a common cytogenetic abnormality characterized by a female phenotype with short stature, gonadal dysgenesis, and various somatic stigmata. The etiology for these specific features is obscure. Evidence points to a lack of important genes on the missing X-chromosome or segment. Circulating androgen levels are reduced in TS and androgen treatment may improve some problems in TS patients. Thus androgen deficiency maybe involved in the pathophysiology of TS. Androgen acts through its intracellular receptor (AR). Polymorphic trinucleotide repeats of CAG can regulate activity of the AR. Changes in CAG repeat number have been implicated in the pathogenesis of spinal bulbar muscular atrophy, ambiguous genitalia, and cancer. We evaluated CAG repeat length of AR in TS. We also evaluated any relation to parental origin of the X-chromosome.

CAG repeat length and X chromosomal parental origin was analyzed in 60 45,X TS patients by means of genotyping and sequencing techniques. CAG repeats were categorized into two groups by the trinucleotide repeat length; short (CAG < 22) and long (CAG  $\geq$  22) repeat length. The frequencies of short and long repeats are 20% (12/60) and 80% (48/60), while in 96 controls 8.3% (8/96) had the short repeat and 91.7% (88/96) had the long repeat. TS patients have a significantly higher frequency of the short CAG repeat compared with normal controls ( $P=0.034$ ). Parental origin of CAG repeats was determined in 49 of 60 TS cases. In 12 patients with short CAG repeat 5 cases had a paternally derived X and 7 patients had a maternally derived X. In 37 patients with long CAG repeat 11 had a paternally derived X and 26 had a maternally derived X. No significant difference in parental origin of the CAG repeat exists between the patients with short and long repeats ( $P>0.05$ ).

Our results show no association between CAG repeat length and parental source of the X chromosome. TS patients appear to have a greater chance of short CAG repeats than controls. This might contribute to some of the phenotypic features of TS.

**Patterns of Differential Gene Expression In Schizophrenia Overlap In Cortical Regions.** *M.P. Vawter<sup>1</sup>, S.J. Evans<sup>2</sup>, P. Choudary<sup>3</sup>, M. Atz<sup>1</sup>, H. Tomita<sup>1</sup>, B. Bolstad<sup>5</sup>, J. Li<sup>4</sup>, T. Speed<sup>6,7</sup>, R. Myers<sup>4</sup>, S.J. Watson<sup>2</sup>, E.G. Jones<sup>3</sup>, H. Akil<sup>2</sup>, W.E. Bunney<sup>1</sup>, NIMH Conte Center and Pritzker Neuropsychiatric Disorders Research Consortium.* 1) Dept Psychiatry, Univ California, Irvine, Irvine, CA; 2) MHRI, University of Michigan, Ann Arbor, MI; 3) Center for Neuroscience, University of California, Davis, CA; 4) Stanford Human Genome Center, Stanford University, Palo Alto, CA; 5) Group in Biostatistics, University of California, Berkeley, CA; 6) Department of Statistics, University of California, Berkeley, CA; 7) Division of Genetics and Bioinformatics, WEHI, Melbourne, Australia.

This project involves a multi-site collaborative effort to investigate gene expression in neuropsychiatric disorders in multiple brain regions. We have investigated schizophrenia (n = 9) and controls (n = 9) by microarray analysis using Affymetrix U133A and B chips. We report on the microarray comparison of three cortical regions: dorsolateral prefrontal cortex (DLPFC), anterior cingulate, and entorhinal cortex (ERC). We selected the top and bottom differentially expressed genes for schizophrenia from a robust probe level linear model analysis (<http://stat-www.berkeley.edu/users/bolstad/AffyExtensions/AffyExtensions.html>) for each brain region. The top 500 and bottom 500 changed genes were further classified using Gene Ontology and KEGG classifications. There was over-representation of ribosomal genes, heat shock related genes, chaperone genes, and immune genes in cortical regions. The preliminary gene classifications for the ERC, DLPFC and anterior cingulate will be compared to the results from the hippocampus. The preliminary microarray evidence suggests several cortical regions share overlapping expression patterns for genes dysregulated in schizophrenia. This work was supported by NIH Conte Center Grant MH60398-03 and the Pritzker Neuropsychiatric Disorder Research Consortium Fund.

**Effects of Mood Disorders and Suicide on Gene Expression Profiles in Postmortem Brains.** *H. Tomita<sup>1</sup>, M.P. Vawter<sup>1</sup>, S.J. Evans<sup>2</sup>, P. Choudary<sup>3</sup>, J. Li<sup>4</sup>, B. Bolstad<sup>5</sup>, T. Speed<sup>6,7</sup>, R.M. Myers<sup>4</sup>, E.G. Jones<sup>3</sup>, S. Watson<sup>2</sup>, H. Akil<sup>2</sup>, W.E. Bunney<sup>1</sup>, NIMH Conte Center and Pritzker Neuropsychiatric Disorders Research Consortium.* 1) Dept Psychiatry, Univ California, Irvine, Irvine, CA; 2) MHRI, University of Michigan, Ann Arbor, MI; 3) Center for Neuroscience, University of California, Davis, CA; 4) Stanford Human Genome Center, Stanford University, Palo Alto, CA; 5) Group in Biostatistics, University of California, Berkeley, CA; 6) Department of Statistics, University of California, Berkeley, CA; 7) Division of Genetics and Bioinformatics, WEHI, Melbourne, Australia.

To examine gene expression changes in mood disorders, gene transcript abundance was measured by microarray analysis in patients with bipolar disorder type I (BPD, n = 9), major depressive disorder (MD, n=11), and compared to a control group (n=20) matched for age, gender, and post mortem interval (PMI). Also, a subgroup of 4 BPD and 6 MD patients who committed suicide was compared to the remaining mood disorder patients, and controls who died rapidly by accident or acute diseases. Total RNA was extracted from three brain regions, anterior cingulate, dorsolateral prefrontal and cerebellar cortices, and each sample was run in duplicate on Affymetrix U95Av2 chips at 3 laboratories. Signal intensity of each gene was extracted using three independent methods, microarray suite version 5.0, robust multi-array analysis (RMA) and probe level model analysis (PLM). Microarray findings were validated by quantitative RT-PCR. Effects of agonal state, brain pH, age, gender, and PMI on gene expression profile were evaluated along with the disorders. These differences in gene expression profile involving multiple signaling pathways such as MAPK pathway will be discussed in the context of pathogenesis of severe mood disorders involving suicidal behavior. This work was supported by NIH Conte grant MH60398-03 and the Pritzker Neuropsychiatric Disorder Research Consortium Fund. We acknowledge participation of all Consortium members in this work.

**Heterozygous mutations in *BBS1*, *BBS2* and *BBS6* have a potential epistatic effect on Bardet-Biedl patients with two mutations at a second BBS locus.** *J.L. Badano*<sup>1</sup>, *J.C. Kim*<sup>2</sup>, *B.E. Hoskins*<sup>3</sup>, *R.A. Lewis*<sup>4,5,6,7</sup>, *S.J. Ansley*<sup>1</sup>, *D.J. Cutler*<sup>1</sup>, *C. Castellan*<sup>9</sup>, *P.L. Beales*<sup>3</sup>, *M.R. Leroux*<sup>2</sup>, *N. Katsanis*<sup>1,8</sup>. 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD 21287, USA; 2) Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby BC, Canada V5A 1S6; 3) Molecular Medicine Unit, Institute of Child Health, University College London, London WC1N 1EH, UK; 4) Departments of Molecular and Human Genetics; 5) Ophthalmology; 6) Pediatrics and; 7) Medicine, Baylor College of Medicine, Houston, TX 77030, USA; 8) Wilmer Eye Institute, Johns Hopkins University; 9) The Clinical Genetics Service, Bolzano General Hospital, Bolzano 39100, Italy.

Bardet-Biedl syndrome (BBS) is a pleiotropic genetic disorder with substantial inter- and intrafamilial variability, that also exhibits remarkable genetic heterogeneity, with seven mapped BBS loci in the human genome. Recent data have demonstrated that BBS may be inherited either as a simple Mendelian recessive or as an oligogenic trait, since mutations at two loci sometimes are required for pathogenesis. This observation suggests that genetic interactions between the different BBS loci may modulate the phenotype, thus contributing to the clinical variability of BBS. We present three families with two mutations in either *BBS1* or *BBS2*, in which some but not all patients carry a third mutation in *BBS1*, *BBS2*, or the putative chaperonin *BBS6*. In each example, the presence of three mutant alleles correlates with a more severe phenotype. For one of the missense alleles, we also demonstrate that the introduction of the mutation in mammalian cells causes a dramatic mislocalization of the protein compared with the wild-type. These data suggest that triallelic mutations are not always necessary for disease manifestation, but might potentiate a phenotype that is caused by two recessive mutations at an independent locus, thus introducing an additional layer of complexity on the genetic modeling of oligogenicity.

**Multiplexed Pyrosequencing Analysis of CARD15 Gene Mutations in Inflammatory Bowel Disease.** *S. Toth*<sup>1</sup>, *O. Palmieri*<sup>2</sup>, *A. Ferraris*<sup>2</sup>, *A. Andriulli*<sup>2</sup>, *A. Latiano*<sup>2</sup>, *V. Annese*<sup>2</sup>, *B. Dallapiccola*<sup>2</sup>, *M. Devoto*<sup>2</sup>, *S. Surrey*<sup>2</sup>, *P. Fortina*<sup>2</sup>.  
1) Pyrosequencing, Inc., Westboro, MA; 2) Thomas Jefferson University, Philadelphia, PA.

Crohn's disease (CD) and Ulcerative colitis (UC), the main types of Inflammatory Bowel Disease (IBD), have a genetic predisposition. Recently, mutations in the Caspase recruitment domain protein 15 (CARD15/NOD2) gene have been identified in patients with Crohn's disease, one of the most frequent causes of gastrointestinal morbidity in Western Europe and North America. A triplex Pyrosequencing protocol was developed for the rapid detection of three common, clinically relevant single-base changes (SNPs) in the NOD2 gene. Pyrosequencing technology was used to detect gene variants: 3020InsC, Arg702Trp, and Gly908Arg in research samples from Italian families and unrelated controls. Three PCR primer pairs were used to amplify the region encompassing each single-base variant interrogated in this study. One primer from each pair contained a 5'-biotin group that was used to purify single-stranded DNA bound to Sepharose beads. Template DNA was annealed with a sequencing primer and the primer strand was elongated by the sequential addition of individual nucleotides to generate sequence information. Following each nucleotide addition and extension step, excess dNTPs were degraded by apyrase. Pyrosequencing technology couples DNA sequencing by synthesis with an enzymatic cascade to generate a light signal whenever pyrophosphate is released following nucleotide incorporation into the DNA template. Research samples from 458 IBD families (with 2+ affected individuals) were analyzed, including 170 CD (affected and non-affected members), 210 UC and 78 Mixed families (members with CD or UC). Genotyping results using Pyrosequencing technology were shown to be concordant with results obtained previously with other genotyping methods. A significant association between NOD2 gene mutations and CD was demonstrated in the Italian population samples. This technology was shown to be a rapid and effective research methodology for genotyping mutations in complex diseases.

**Translocation breakpoint in two unrelated Tourette syndrome cases, within a region previously linked to the disorder.** *F. Crawford*<sup>1,2</sup>, *G. Ait-Ghezala*<sup>1</sup>, *M. Sutcliffe*<sup>3</sup>, *R. Hauser*<sup>4</sup>, *A. Silver*<sup>1</sup>, *M. Mullan*<sup>2,5</sup>. 1) Child Development Center, Department of Psychiatry, Univ South Florida, Tampa, FL; 2) James A Haley Veterans Administration, Tampa, FL; 3) All Childrens Hospital, St. Petersburg, FL; 4) Department of Neurology, University of South Florida, Tampa, FL; 5) Roskamp Institute, 2040 Whitfield Avenue, Sarasota, FL.

We have described two unrelated families wherein balanced t(6;8) chromosomal translocations occur in individuals diagnosed with Tourette Syndrome (TS). In one of these families, the transmission of the translocation is associated with learning and behavioral difficulties; in the other family one parent is unaffected and the other cannot be traced, thus transmission cannot be demonstrated and it is possible that the translocation may have occurred de novo. The breakpoint on chromosome 8 occurs within the q13 band in both families, suggesting that a gene or genes in this region might contribute to the TS phenotype. Existing linkage and cytogenetic data, suggesting involvement of chromosome 8 in TS families and individuals, further support this hypothesis. We have identified two YAC clones mapping distal and proximal to the chromosome 8 translocation site as determined by Fluorescent In Situ Hybridization (FISH). PCR amplification of genetic markers in this region, using isolated chromosomes from one of the patients, followed by BAC screening with the closest flanking genetic markers, has identified a 200Kb BAC which by FISH we have demonstrated encompasses the chromosome 8 breakpoint in both families. The fact that the chromosomal breaks in the TS cases from both families occur within such a small region of chromosome 8 further supports the hypothesis that disruption of a gene or genes in this part of chromosome 8 contributes to the clinical phenotype in these families.

**Functional COMT Polymorphism in a 22q11 Deletion Syndrome adult population.** *M. Gheorghiu<sup>1</sup>, E.W.C. Chow<sup>1,2</sup>, R. Weksberg<sup>2,3</sup>, A.S. Bassett<sup>1,2</sup>, O. Caluseriu<sup>1,3</sup>.* 1) Clinical Genetics Research, CAMH, Toronto, Ontario, Canada; 2) University of Toronto; 3) Hospital for Sick Children.

22q11 Deletion Syndrome (22qDS) is a congenital, autosomal dominant syndrome characterized by variable clinical features including learning disabilities, palatal abnormalities, congenital cardiac defects and psychiatric disorders, mainly schizophrenia. A microdeletion of 1.5-3.0 Mb is detectable by FISH in the majority of 22qDS patients but genetic mechanisms underlying the pathogenesis of 22qDS still remain to be clarified. One of the genes mapped to 22q11.2 is catechol-O-methyltransferase (COMT), an enzyme involved in degradation of dopamine. COMT has been proposed as a candidate for expression of psychiatric disorders including schizophrenia because of the role dopamine is hypothesised to play in schizophrenia pathogenesis. The COMT gene contains in exon 4 a functional polymorphism, Val158/108Met, that determines high or low activity of this enzyme, respectively. This could influence the level of dopamine in the prefrontal lobe which may be involved in the cognitive impairment and other behavioural expression commonly found in schizophrenia. We genotyped 50 adult subjects with 22qDS, 25 with schizophrenia (22qDS-SZ) and 25 non-psychotic (22qDS-NP), in order to investigate the Val158/108Met functional polymorphism in a COMT hemizygous population. There was no significant difference between the 22qDS-SZ and 22qDS-NP groups (n=13 and n=12, respectively, with the Met allele). However, examining symptom expression in the overall sample who had detailed symptom data we found that the 22 subjects (n=12 22qDS-SZ; n=10 22qDS-NP) with the Met allele had significantly more severe ratings on the excitement factor of schizophrenia than the 24 subjects (n=11 22qDS-SZ; n=13 22qDS-NP) with the Val allele ( $p=0.003$ ). Excitement factor items include hostility and impulsivity. These findings are consistent with a minor role of Val158/108Met as a modulator factor involved in schizophrenia pathogenesis in 22qDS patients.

**A balanced translocation t(4;15) associated with severe obesity.** *J. Klar*<sup>1</sup>, *B. Åsling*<sup>2</sup>, *M. Ulvsbäck*<sup>2</sup>, *A. Dellsén*<sup>2</sup>, *C. Ström*<sup>2</sup>, *A. Forslund*<sup>3</sup>, *N. Dahl*<sup>1</sup>. 1) Dept Genetics & Pathology, Uppsala Univ, Uppsala, Sweden; 2) AstraZeneca R&D, Mölndal, Sweden; 3) Department of Medical Sciences, Uppsala University, Sweden.

Obesity is a highly prevalent, multigenic trait that predicts increased morbidity and mortality and contributes to many health problems in the Western society. We have identified a family in which a mother and her two children present with severe obesity. These individuals have a body mass index (BMI) of 43-50 (normal 19-25). Chromosome analysis revealed a balanced reciprocal translocation t(4;15) in affected individuals. Dietary factors behind the phenotype were excluded. Clinical and biochemical investigations did not reveal any associated abnormalities or metabolic disturbances. Here we report the molecular cloning of the breakpoints and the identification of two genes affected by the rearrangement on chromosome 4 and 15 respectively. A novel fusion gene is formed by the translocation and a corresponding fusion transcript with an ORF is detected in adipocytes. This translocation and the identification of genes possibly affected by the breakpoints lead to the identification of yet unknown obesity-related genes and pathways.

**Behavioral characterization of mouse models for Smith-Magenis syndrome and Dup 17 (p11.2 p11.2).** *K. Walz<sup>1</sup>, C. Spencer<sup>1</sup>, K. Kaasik<sup>1</sup>, C.C. Lee<sup>1</sup>, J.R. Lupski<sup>1,2,3</sup>, R. Paylor<sup>1,4</sup>*. 1) Molecular & Human Gen, Baylor College of Medicine, Houston, TX; 2) Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Childrens Hospital, Houston, Texas; 4) Division of Neurosciences, Baylor College of Medicine.

Contiguous gene syndromes (CGS) refer to a group of disorders associated with chromosomal rearrangements in which the phenotype is thought to result from altered copy number of physically linked dosage sensitive genes. Smith Magenis Syndrome and [dup(17)(p11.2p11.2)] are CGS associated with a deletion or a duplication of band p11.2 of chromosome 17 respectively. Previously we reported the creation, by chromosomal engineering, of rearranged chromosomes carrying a deletion/deficiency (Df(11)17) or a duplication (Dp(11)17) of the syntenic region on mouse chromosome 11 that spans the genomic interval commonly rearranged in these patients. Here we present an exhaustive behavioral analysis of these models indicating that heterozygous animals carrying the engineered deletion (Df(11)17/+) or the duplication (Dp(11)17/+) are hypoactive or hyperactive respectively and both have learning disabilities. In addition period length differences were found for (Df(11)17/+) mice compared with wt littermates. These results indicate that a dosage sensitive gene present in this region is responsible for behavioral abnormalities in the mouse as has been shown for the human syntenic interval.

**Identification of Chromosome 7 Breakpoints in an Autistic Family Indicates Candidate Genes for Autism**

**Susceptibility.** *D.A. Skaar<sup>1</sup>, L. Christ<sup>2</sup>, M.L. Cucarro<sup>1</sup>, J.R. Gilbert<sup>1</sup>, S. Schwartz<sup>2</sup>, M.A. Pericak-Vance<sup>1</sup>.* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Dept. of Genetics, Case Western Reserve University, Cleveland, OH.

During ascertainment of multiplex families for linkage analysis of autism, a paracentric inversion in Chromosome 7 ((inv(7)(q22-q31.2)) was identified in Family 7543. Two of three children were affected with autism, and the third, while not meeting criteria for autism, exhibits expressive language disorder. All three sibs carry the paracentric inversion, which they inherited from their mother. Analysis of microsatellite markers on 7q showed support for linkage to autism in multiple studies over the ~30cM region which includes this inversion. The evidence for involvement of this inversion in autism is strong, given its occurrence in multiple autistic siblings, and the linkage analysis on this area of 7q. Therefore, the breakpoints of this inversion should identify genes involved in autism.

We report here the identification of the breakpoints. The first identification, to regions of BAC inserts by FISH, identified a 194kb region for the proximal, and a 175kb region for the distal breakpoint. The proximal breakpoint region contains a number of genes and pseudo-genes for Cytochrome P450 polypeptides (CYP3A5, CYP3A5P1, CYP3A7, CYP3A5P2, and CYP3A4) as well as the uncharacterized FLJ32468. The distal breakpoint region contains no known genes, but does contain several transcribed regions, indicated by ESTs, and one exon of a predicted novel gene. In order to identify the genes directly affected, the location of breakpoints is being narrowed down by southern blotting of restriction fragments, which will be presented.

**Serum cholesterol and suicidality in Smith-Lemli-Opitz syndrome heterozygotes.** A. Lalovic<sup>1</sup>, L. Merkens<sup>2</sup>, G. Arsenault-Lapierre<sup>1</sup>, M. Nowaczyk<sup>3</sup>, F.D. Porter<sup>4</sup>, L. Russell<sup>5</sup>, R. Steiner<sup>2</sup>, G. Turecki<sup>1</sup>. 1) McGill Group for Suicide Studies, Douglas Hospital Research Centre, Verdun, QC, Canada; 2) Oregon Health Sciences University, Portland, OR, USA; 3) McMaster University, Hamilton, ON, Canada; 4) Heritable Disorders Branch, NICHD, NIH, Bethesda, MD, USA; 5) Montreal Children's Hospital, Montreal, QC, Canada.

It is well established that family history of suicidal behavior represents an increased risk of suicide for an individual, and this combined with consistent evidence from twin and adoption studies suggests that there is a genetic component to suicidal behavior. Interest in cholesterol as a biological marker of suicide risk has been sparked by a large number of studies demonstrating an association between low serum cholesterol and suicidal behavior. Smith-Lemli-Opitz syndrome (SLOS) heterozygotes may serve as an interesting model in which to examine the relationship between natively lower levels of cholesterol and suicidal behavior, while concurrently investigating the genetic aspects of suicide. Smith-Lemli-Opitz syndrome is a developmental disorder caused by recessively inherited mutations in the gene for the enzyme 7-dehydrocholesterol reductase involved in cholesterol biosynthesis. Parents of SLOS children are obligate heterozygotes and are clinically normal, but due to the partial enzymatic defect have cholesterol levels that fall in the lower range. We examined suicidal behavior, impulsivity, aggression and depression in SLOS heterozygotes (n = 51) and controls (n = 54). Controls, like SLOS heterozygotes, were parents of children with a chronic debilitating disorder, thus controlling for important effects of care-giver stress. SLOS heterozygotes reported a positive family history of attempted and completed suicide significantly more frequently than controls (30.6% SLOS heterozygotes, 9.3% controls;  $p < 0.05$ ), while family history of psychiatric disorders did not significantly differ between the groups. History of attempted suicide tended to be more common among SLOS heterozygotes (6.0% SLOS heterozygotes, 1.9% controls;  $p > 0.05$ ). Behavioral measures are discussed in the context of these findings.

**Mutations in PTCH maybe rare causes of non-syndromic cleft lip and palate.** *M. Mansilla*<sup>1</sup>, *E. Castilla*<sup>2,3</sup>, *J. Lopez Camelo*<sup>4</sup>, *J. Murray*<sup>1</sup>. 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Dept of Genetics, Fiocruz, Brazil; 3) CONICET, Argentina; 4) IMBICE, Buenos Aires, Argentina.

Patched (PTCH) is the proposed receptor for sonic hedgehog (SHH) in a complex with smoothened (SMO) and regulates the expression of WNT and TGF beta. Mutations in PTCH cause the nevoid basal cell carcinoma syndrome (NBCCS) which include rib and craniofacial abnormalities and multiple tumor types. Cleft palate is found in 4% of the cases. To determine if mutations in PTCH might cause non-syndromic forms of cleft, we sequenced the complete coding sequence and exon/intron boundaries in the 23 exons of PTCH in DNA samples from 180 probands with non-syndromic cleft lip and palate. We detected 3 new missense mutations and for these exons we sequenced all family members available, an additional 180 cleft cases and 90 normal controls from the same region. In exon 6 (extracellular domain) we found a P295S change. It was present in an affected Filipino female and also in her two non-affected brothers and father. This residue is conserved in mouse, zebrafish and chicken. For exon 9 a D436N change was located in the transmembrane domain and is conserved in chicken, zebrafish and mouse. We found this variation in a case from Iowa and in his unaffected mother, neither of whom on repeat clinical exam had any features of NBCCS. Another change was found in an affected male from the Philippines, with his normal mother showing the same variation. This S827G change was found in exon 15, an extracellular domain. None of the changes were found in 180 controls and there was no history or physical evidence for features of Gorlin Syndrome in any of these 3 affected or in the family members. We propose that mutations in PTCH in the extracellular domain (that is proposed to interact with SHH) and the intracellular domain (proposed to interact with SMO) may interfere with this pathway decreasing SHH activity contributing to clefting. Since the SHH path also includes interactions with cholesterol metabolism, which also results in defects that cause cleft palate, this phenotypic feature may arise from complex interactions of genes and environmental influences.

**Variants in SKI, IRF6, and RFC1 are associated with cleft lip/palate in a South American population. A.R.**

*Vieira*<sup>1</sup>, *I.M. Orioli*<sup>2</sup>, *E.E. Castilla*<sup>3</sup>, *M.E. Cooper*<sup>4</sup>, *M.L. Marazita*<sup>4</sup>, *J.C. Murray*<sup>1</sup>. 1) Pediatrics, The University of Iowa, Iowa City, IA; 2) ECLAMC at Department of Genetics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; 3) ECLAMC at Department of Genetics, Oswaldo Cruz Institute, Rio de Janeiro Brazil and CEMIC, Buenos Aires, Argentina; 4) Center for Craniofacial and Dental Genetics, University of Pittsburgh, Pittsburgh, PA.

ECLAMC (Estudio Colaborativo Latino Americano de Malformaciones Congenitas or Latin American Collaborative Study of Congenital Malformations) is a program which has investigated the causes of congenital malformations and their frequencies in Latin American hospitals since 1967. Beginning in January 1998, ECLAMC has collected biological samples from children with nonsyndromic oral-facial clefts (NSOFC) and their mothers for molecular analysis. NSOFC are common congenital malformations known to occur in approximately 1/1,000 live births in ECLAMC hospitals. The present study used the likelihood ratio test (LRT)-based analysis to detect non-Mendelian transmission of DNA sequence variants in IF6, SKI, RFC1, MTHFR, TGFA, and TGFB3 to 233 South American children from their respective mothers. The results show association between NS cleft lip (CL) and SKI ( $p=0.004$ ) and weaker association with NS cleft lip with or without cleft palate (CL/P) ( $p=0.042$ ). Previous studies have shown that the frequency of mitochondrial haplotype D (HapD) among these clefting cases is higher than the general population. When the analysis was divided by children with HapD versus any other haplotype group, a borderline difference between these two groups were seen for SKI ( $p=0.09$ ), but significant differences were seen for IRF6 and RFC1 ( $p=0.003$ ). An association between CL/P and IRF6 ( $p=0.023$ ), and CL and RFC1 ( $p=0.017$ ) was seen only for the non HapD group. These results support previous linkage and linkage disequilibrium findings with 1p36, 1q32, and 4p in humans and suggest that SKI, IRF6, and RFC1 mutations make a contribution to clefts in South American populations, however these contributions will depend on the type of Amerindian ancestry.

**Molecular and cytogenetic analyses in Brazilian patients affected by autism spectrum disorders.** *E.S. Moreira<sup>1</sup>, G.M. Orabona<sup>1</sup>, L.O. Avelar<sup>1</sup>, E. Vadasz<sup>2</sup>, L.M. Biason<sup>2</sup>, V.N.O. Takahashi<sup>1</sup>, K. Abe<sup>1</sup>, A.M. Vianna-Morgante<sup>1</sup>, M.R. Passos-Bueno<sup>1</sup>.* 1) Centro de Estudos do Genoma Humano/Departamento de Biologia/ Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, Brazil; 2) SEPIA, Instituto de Psiquiatria, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brazil.

Autism spectrum disorders (ASDs) comprise a group of neurodevelopmental conditions characterized by impairments in reciprocal communication and social interaction, as well as by unusually restricted and stereotyped patterns of behaviors and interests. The symptoms are usually apparent in the first three years of life and the population prevalence has been estimated in 1/1000, with a sex ratio of 4:1 male to female. Although most cases of ASDs seem to be caused by complex genetic mechanisms, 10 to 15% appear in association with chromosomal abnormalities or monogenic conditions. Chromosomal abnormalities among autistic individuals have been reported with frequencies from 5 to 12%. The FMR1 (CGG)<sub>n</sub> expansion typical of the fragile X syndrome has been found in up to 13% ASD patients. Two recent papers described the identification of pathogenic mutations in the gene MECP2 in three girls with autism. In our study, we selected 126 Brazilian families with ASDs, using the diagnostic criteria outlined in DSM-IV, to search for chromosomal abnormalities and/or mutations in the FMR1 and MECP2 genes. These 28 female and 108 male patients are grouped in 116 sporadic and 10 familial cases. No chromosomal alterations were detected in 80 individuals after G-banding analysis. Among the 100 male cases tested for the FMR1 (CGG)<sub>n</sub> expansion through PCR, only one was found to carry the full mutation. This result was further confirmed through Southern blotting. No mutations were detected in the 90 individuals screened for the exons 2 and 3 of the MECP2 gene through denaturing high-performance liquid chromatography (dHPLC). We are currently analyzing exon 4 of MECP2. Although preliminary, these data suggest that chromosomal alterations and FMR1 mutations are not a significant factor in the etiology of ASDs in Brazil. Financial support: FAPESP, CNPq, CEPID.

**Identification of a celiac disease gene on chromosome 19.** *M. van Belzen*<sup>1,2</sup>, *A. Zhernakova*<sup>1</sup>, *A. Bardoel*<sup>1</sup>, *R. Houwen*<sup>2</sup>, *C. Mulder*<sup>3</sup>, *C. Wijmenga*<sup>1</sup>. 1) Biomedical Genetics, UMCU, Utrecht, Netherlands; 2) Pediatric Gastroenterology, UMCU, Utrecht, Netherlands; 3) Department of Gastroenterology, VU University Medical Center, Amsterdam, Netherlands.

Celiac disease is a common autoimmune disorder and is strongly associated to HLA-DQ2 and DQ8. However, the contribution of the HLA-region has been estimated at approximately 40%, so non-HLA genes must also be involved in the etiology of celiac disease. We have recently performed a genomewide screen in 101 affected sibpairs to localize these genes, and we identified significant linkage to 19p13.1 (MMLS 4.31; nominal  $p = 6.2 \times 10^{-6}$ ). The size of the maximum LOD-1 candidate region was only 3 Mb, but this region is very gene-rich and contained 92 genes and ESTs. Therefore we performed fine-mapping of the region in a case-control cohort of 216 celiac disease patients and 216 matched controls to identify the celiac disease susceptibility gene. Firstly, the region was saturated with microsatellite markers and one marker was significantly associated with celiac disease ( $p = 0.0013$ ). Secondly, 50 single nucleotide polymorphisms (SNPs) were typed using a DNA-pooling strategy, and four SNPs in a 30 kb region showed significant association. Single typing of these SNPs confirmed these findings (individual  $p < 0.01$ ). The four associated SNPs combine into a single haplotype that is more frequent in cases versus controls ( $p = 0.012$ ). Although this gene has no obvious role in intestinal or autoimmune processes, its expression pattern is consistent with intestinal function. Implication of this gene in the etiology of celiac disease may lead to the identification of a pathway not previously implicated in celiac disease pathogenesis.

**The MAOA promoter polymorphism and disruptive behavior disorders: Gene-environment interaction.** *M.M. Vanyukov*<sup>1,2,3,5</sup>, *B.S. Maher*<sup>1,4,5</sup>, *B. Devlin*<sup>1,3,5</sup>, *R.E. Tarter*<sup>1,3,5</sup>, *L. Kirisci*<sup>1,3,5</sup>, *G.P. Kirillova*<sup>1,5</sup>, *R.E. Ferrell*<sup>1,2,5</sup>. 1) Center for Education and Drug Abuse Research (CEDAR), Dept. of Pharmaceutical Sciences; 2) Dept. of Human Genetics; 3) Dept. of Psychiatry; 4) School of Dental Medicine; 5) University of Pittsburgh, Pittsburgh, PA.

Monoamine oxidase A (MAO-A) is a primary candidate for testing its contribution to variation in the risk for behavior disorders because of its important role in the metabolism of neurotransmitters, including dopamine and serotonin. A point nonsense mutation inactivating the *MAOA* gene results in a behavioral syndrome including high aggression (Brunner et al., *Science*, 262:578-580, 1993). Our findings (Vanyukov et al., *Am J Hum 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). Caspi et al. (*Science*, 297:851-854, 2002) reported that a functional *MAOA* promoter polymorphism moderated the relationship between indices of maltreatment in children and their later antisocial behavior. To extend these findings, we tested the relationship between the *MAOA* promoter polymorphism, indices of paternal and maternal neglect and the risk for disruptive behavior (conduct, oppositional defiant, and attention deficit hyperactivity) disorders in a longitudinal study of a sample of European-American males, using regression, correlation and survival analyses. Similar to the Caspi et al. results, the strength of association between neglect and behavioral disorders (CD and ADHD) depended on the *MAOA* alleles. Their role, however, was reverse to that observed by Caspi et al. for maltreatment. Significant relationships, both with the risk of the disorder and the rate of its development, were detected only in the group with 3.5- and 4-repeat ("high activity") alleles. These findings support involvement of variation in the *MAOA* gene in the development of behavioral deviation.

**Single nucleotide polymorphisms of the human GFPT1 gene are associated with type 2 diabetes and diabetic nephropathy.** *Y. Jia<sup>1</sup>, H. Zhang<sup>1</sup>, Z. Zhang<sup>1</sup>, T. Hale<sup>1</sup>, J. Cooper<sup>1</sup>, S.C. Elbein<sup>1,2</sup>.* 1) Medicine, University of Arkansas for Medical Sciences, Little Rock, AR; 2) Central Arkansas Veterans Healthcare System, Little Rock, AR 72205.

Considerable data support a role of increased glucose flux through the hexosamine pathway in insulin resistance, defective insulin secretion, and diabetes complications including diabetic nephropathy. Glucose flux into this pathway is controlled by the enzyme glutamine:fructose-6-phosphate amidotransferase (GFPT), which is encoded by separate genes from unlinked genetic loci, GFPT1 and GFPT2. To examine the role of GFPT1 variants in the susceptibility to type 2 diabetes (T2DM) and diabetic nephropathy (DN), we screened a total of 7170 bp of sequence, including 1 kb of 5' flanking sequence, 5' and 3' untranslated regions, all 19 exons, and 100 bp to 200 bp of sequence flanking each exon for mutations by DHPLC in 24 Caucasian (C) and 24 African American (AA) individuals. We identified 9 single nucleotide polymorphisms (SNPs), of which 7 were found in both populations, and 2 were found only in the African American population. One SNP was in the putative promoter region, 2 SNPs were in the 3'UTR and the remaining SNPs were either intronic or flanking. We typed the 6 common SNPs in 192 Caucasian individuals with diabetes and 192 control individuals. One SNP in intron 9 is associated with diabetes in Caucasians ( $p=0.0435$ ). Seven SNPs were typed in 149 AA with DN, 113 AA T2DM without DN, and 88 AA controls. One SNP in intron 1 and one SNP in 3' UTR were associated with DN ( $p=0.0102$  and  $p=0.0141$ ), but no SNP was associated with T2DM. Real time RT-PCR analysis showed a 30% increase in mRNA levels in transformed lymphocytes from T2DM individuals and a 2-fold increase in DN individuals in Caucasian population. No significant differences were found in AA T2DM and DN individuals, and no difference in allele-specific expression was seen for the 3' untranslated SNP. SNPs in GFPT 1 were associated with T2DM and DN. Our data suggest that increased GFPT1 expression resulting from common SNPs may cause increased glucose flux through the hexosamine pathway and contribute to T2DM and diabetic nephropathy.

**Susceptibility to polycystic ovary syndrome (PCOS) is associated with variation in the region of the insulin receptor gene.** *D. Stewart*<sup>1</sup>, *M. Urbanek*<sup>2</sup>, *T. Smith*<sup>1</sup>, *W. Ankener*<sup>1</sup>, *K. Ewens*<sup>1</sup>, *R. Legro*<sup>3</sup>, *J. Strauss*<sup>1</sup>, *A. Dunai*<sup>2</sup>, *R. Spielman*<sup>1</sup>. 1) U of Pennsylvania Sch of Medicine, Phila. PA; 2) Feinberg Sch of Medicine, Northwestern U, Chicago, IL; 3) Penn State U Sch of Medicine, Hershey, PA.

Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility. Although familial aggregation is observed, inheritance is non-Mendelian and PCOS is considered a complex trait. In previous studies of markers at or near 37 candidate genes, we found evidence for both linkage and association at only one marker: D19S884, located approximately 1 Mb centromeric to the insulin receptor (*INSR*) on chromosome 19. The mean sharing for this marker in 98 affected sib pairs was 63% ( $p=0.004$ ), and the transmission/disequilibrium test (TDT) chi-square for allele 8 is 11.85 ( $p=0.0006$ ) (all  $p$ -values are nominal). *INSR* is of special interest because insulin resistance is a hallmark of PCOS and PCOS-like features (hirsutism, hyperandrogenemia and polycystic ovaries) are seen in some individuals with the syndromes of extreme insulin resistance secondary to mutations in *INSR*. We have now analyzed data for SNPs in this region from approximately 400 families. For 11 SNPs (10 intronic and one exonic) distributed throughout *INSR*, we found no TDT values greater than 1.2 ( $p > 0.5$ ). We have studied two intronic SNPs located close to D19S884; for one (1.9 kb from D19S884), the TDT was 4.7 (marginally significant:  $p=0.03$ ). At the other SNP (6.2 kb from D19S884 in the opposite direction), the TDT was 0.198 (NS). Comparison of DNA sequence in this intronic region as provided by the UCSC Genome Browser shows homology between mouse and human; this finding is consistent with a possible regulatory role in gene expression. The lack of evidence for association among 11 SNPs in *INSR* makes it unlikely that variation in *INSR* itself accounts for susceptibility to PCOS. The presence of a SNP with modest association 1.9 kb from D19S884 strengthens the evidence that genetic variation in that immediate region is associated with PCOS. The evidence for conserved non-coding sequence supports the possibility of functional elements in this region that affect susceptibility to PCOS.

**Polymorphism in the androgen receptor and mammographic density in women taking and not taking estrogen and progestin therapy.** *E.O. Lillie<sup>1,2</sup>, L. Bernstein<sup>1</sup>, S.A. Ingles<sup>1</sup>, W.J. Gauderman<sup>1</sup>, G. Rivas<sup>2</sup>, V. Gagalang<sup>2</sup>, T. Krontiris<sup>2</sup>, G. Ursin<sup>1,2,3</sup>.* 1) Keck School of Medicine, University of Southern California, Los Angeles, CA 90089; 2) City of Hope/Beckman Research Institute, Duarte, CA 91010; 3) University of Oslo, Norway.

Some evidence exists indicating that women with the less active form of the androgen receptor (*AR*) genotype, characterized by a higher number of CAG repeat lengths, have increased breast cancer risk. Mammographic density is a strong breast cancer risk factor that probably reflects hormone induced cell proliferation in the breast. We evaluated the association between *AR*-CAG repeat length and mammographic density. We genotyped blood samples and obtained mammographic density information from 404 African-American and Caucasian breast cancer patients in Los Angeles County who participated in a population-based case-control study. We used the median *AR*-CAG length as the cut-point between short (S) and long (L) CAG repeat length. To model the dependence of percent mammographic density on *AR* genotype, we fit linear and ordinal logistic regression models. We observed no association between *AR*-CAG length and mammographic density among all women combined, among premenopausal women, or among postmenopausal women with no estrogen-progestin therapy (EPT) use. In postmenopausal EPT users, carriers of the less active *AR*-CAG (L/L genotype) had statistically significantly higher mean percent density (36.8%) than carriers of the more active *AR*-CAG (S/S genotype) (16.9%,  $p=0.01$ ). In this group, the odds ratio (OR) for a single quartile increase in percent mammographic density per long allele was 4.73; (95% confidence interval (CI): 1.89-11.79,  $p$  trend $<0.0001$ ). Our results suggest that while the *AR* gene itself is not a strong predictor of mammographic density, it is associated with increased mammographic density in women on EPT. The *AR* gene may therefore play a key role in determining whether EPT causes breast cancer in women.

**Genotypic variation of specific SNPs in the Calpain 10 gene within a cohort of the Black South African**

**population.** *A. Olckers*<sup>1,2</sup>, *G.W. Towers*<sup>1</sup>, *M.N. Wessels*<sup>1</sup>, *P. Rheeder*<sup>3</sup>, *P. Schwarz*<sup>4</sup>. 1) Centre for Genome Research, Potchefstroom University, Pretoria, South Africa; 2) DNAbiotec (Pty) Ltd, Pretoria, South Africa; 3) Dept of Clinical Epidemiology, University of Pretoria, South Africa; 4) Department of Endocrinopathies and Metabolic Diseases, Medical Faculty Carl-Gustav-Carus, Technical University Dresden, Germany.

Type 2 diabetes mellitus (T2D) is a term utilised to describe a large group of phenotypically heterogenous metabolic disorders having numerous aetiologies characterised by chronic hyperglycaemia and disturbances in the metabolism of fat, protein and carbohydrates due to insulin loss, insulin insensitivity or both. A major T2D susceptibility locus namely NIDDM1 was localised to chromosome 2 in the Mexican American and Northern European populations. The gene expressing the protein calpain 10 (CAPN10) is located at this specific locus and is an ubiquitously expressed non lysosomal cysteine protease. Various single nucleotide polymorphisms (SNPs) found in the intronic regions of this gene have been associated with T2D in the Mexican American and Northern European populations. Blood samples were collected from 200 clinically well-characterised adult Black South African diabetic patients and 100 Black controls. These individuals underwent molecular screening via real time PCR utilising hybridisation probe technology. The results were utilised in determining the allele and haplotype frequencies of the aforementioned SNPs. Upon comparison to various reported non-African populations it was elucidated that this group of individuals presented with a great amount of variation. The alteration UCSNP-63 was determined not to be in Hardy Weinberg equilibrium within this population, which may be an indication of it being in linkage disequilibrium with T2D. Haplotype analysis detected the absence of various non-African at-risk haplotypes, which may be indicative that the role of this gene in T2D susceptibility is different in the African and non-African populations. The investigation underscores a genetically heterogenous aetiology of T2D susceptibility within the Black South African population.

**Association between TaqI polymorphism of gap junction beta 5 gene (GJB5) and psychotic symptoms in bipolar disorder.** X. Ni<sup>1</sup>, J. Kennedy<sup>1, 2</sup>. 1) Neurogenetics Section, Ctr Addiction & Mental Health, Toronto, ON, Canada; 2) Department of Psychiatry, University of Toronto.

Using differential screening of gene initiation sequences, we found one of the differential DNA signals in the probands with schizophrenia and bipolar disorder compared with their parents in pooled DNAs. The sequence of this DNA fragment showed high homology with the gap junction beta 5 gene (GJB5). Therefore, we selected GJB5 as a candidate gene for major psychotic disorders. Recently, we presented a positive association between the TaqI polymorphism of GJB5 and schizophrenia in 2003 International Conference on Schizophrenia Research. To test for the presence of association between GJB5 and psychotic symptoms in bipolar patients, we analyzed the TaqI polymorphism of GJB5 in 145 BP probands with psychotic symptoms, 133 BP patients without psychosis, and 231 unrelated healthy controls. All cases and controls were collected in Toronto and central Canada. Case-control analysis was performed on the genotype data. The distributions of GJB5 TaqI genotypes in all three groups we tested were in Hardy-Weinberg Equilibrium (HWE). Though there was no significant difference of genotype frequencies between BP probands without psychosis and healthy controls ( $\chi^2=1.3748$ ,  $df=2$ ,  $p=0.5029$ ), we detected a trend to significance between BP probands with and without psychotic symptoms ( $\chi^2=5.0854$ ,  $df=2$ ,  $p=0.0787$ ), and a significant association between the TaqI polymorphism of GJB5 and bipolar psychotic symptoms compared with controls ( $\chi^2=8.118$ ,  $df=2$ ,  $p=0.0173$ ). These results indicate that GJB5 may play a role in the etiology of psychotic symptoms in bipolar disorder. Keywords: bipolar disorder, psychotic symptom, genetics, association analysis, gap junction beta 5 gene. This research was supported by CIHR/CPRF Partnered Fellowships.

Program Nr: 2063 from 2003 ASHG Annual Meeting

**Dopamine System Genes and Human Motor Activity.** *B.S. Maher, M.M. Vanyukov, M.L. Marazita, R.E. Ferrell.*  
Univ Pittsburgh, Pittsburgh, PA.

The dopamine system is hypothesized to play a major role in variation in human activity levels. We applied a measured-genotype approach to investigate the association of polymorphisms at several dopamine system genes with measured motor activity in a population of 103 10-12 year old Caucasian males. Motor activity was measured using an acceleration sensitive wrist-worn monitor worn by each subject during a standardized two-day research protocol. A VNTR polymorphism in the 3' UTR region of the dopamine transporter (DAT1) was significantly associated, without Bonferroni correction, with motor activity. Polymorphisms in the dopamine receptors D1, D2, D4 and D5 were not associated with motor activity.

**Stability of polymorphic trinucleotide repeats of the androgen receptor in uterine leiomyoma.** *J. Yankowitz, S.M. Zeng.* Dept OB/GYN, University of Iowa Roy J. and Lucille A. College of Medicine, Iowa City, IA.

The human androgen receptor gene (AR) contains three polymorphic trinucleotide repeats: CAG, GGN and ACG. These repeats may regulate transactivation activity of the AR. The expansions or reduction of the former two repeats have been implicated in the pathogenesis of several diseases such as spinal bulbar muscular atrophy, ambiguous genitalia, and prostate, breast and colon cancer. Instability of microsatellite repeats have been seen in some neoplasms compared to normal tissue. Uterine leiomyoma are the most common benign uterine tumor occurring in about 25% of women during their lifetime. Little is known about the etiology and pathogenesis of this neoplasia. We evaluated whether the stability and length of these three trinucleotide repeats is associated with uterine leiomyoma. We used PCR and 6% polyacrylamide gel electrophoresis to evaluate the number of triplet repeats in 38 leiomyoma tissues and 20 surrounding normal myometrium. The stability of the trinucleotide repeat was confirmed through the comparison of the genotypes between the tumor and its surrounding normal tissues, and the repeat length of these polymorphisms was determined by directly sequencing. No change in copy number of these three repeats was found in 20 patients with tumor and the corresponding surrounding normal tissue. This indicated that instability of AR trinucleotide repeats may not be involved in the pathogenesis of uterine leiomyoma.

**Screening of an Azorean population with major psychoses at the triplet repeat expansion associated loci SCA8, SCA17 and SCA1.** *T. Fortune*<sup>1</sup>, *C.N. Pato*<sup>2</sup>, *M.T. Pato*<sup>2</sup>, *M.H. Azevedo*<sup>3</sup>, *J.L. Kennedy*<sup>1</sup>, *J.B. Vincent*<sup>1</sup>. 1) Clarke Division, CAMH, Toronto; 2) Center for Psychiatric and Molecular Genetics, SUNY, Syracuse, NY, USA; 3) (3) Department of Psychiatry, University of Coimbra Hospital, Coimbra, Portugal.

The molecular mechanism associated with the anticipation observed in neurodegenerative disorders was identified in 1991 as a triplet repeat expansion (TRE). This has led to the study of polymorphic triplet repeat loci in the context of major psychoses. SCA8 is unusual among the expanded repeat neurodegenerative disorders because not all persons with the TRE develop ataxia. Investigation of unrelated psychosis cases versus controls has found that twice as many expansions (100-1300 repeats) occur at SCA8 in major psychosis cases (1.25%) as unaffected controls (0.7%). We have yet to identify ataxia among psychosis patients with large SCA8 repeat alleles. The SCA8 associated TRE is located in its most 3'exon. This TRE could also influence the expression of Kelch-like 1, KLHL1, which is expressed brain specifically. The most 5' exon of SCA8 overlaps the first exon of KLHL1 which is transcribed in the opposite orientation. Recent studies of ataxic patients with expansions at other known TRE loci has revealed a subgroup of individuals who also have an expansion at SCA8, leading to the speculation that TRE at SCA8 may have a trans acting role in repeat instability. We have previously screened 32 SCZ and BPAD families from the Azores Islands at the SCA8 locus, we have now added a further 70 families. Among the newly examined families we have identified 7 expanded repeats in 6 families. We have screened the same population at the CAG polymorphism at SCA1 and SCA17 loci. All of the SCA17 alleles fell within the normal range, 25-42 CAG repeats and only 2.5% fell outside the most common allele range reported in the normal population, 32-39 CAG repeats. The repeats found at the SCA1 locus were also found to fall within the expected normal range, 6-39 repeats, in fact they were between 10-24 repeats in size. We are screening further members of these families for the SCA8 TRE in order to investigate links between TRE and psychoses.

**Association of SNPs in the ADH gene cluster with alcoholism.** *T. Foroud<sup>1</sup>, D. Dick<sup>1</sup>, X. Xuei<sup>1</sup>, A. Goate<sup>2</sup>, B. Porjesz<sup>3</sup>, H. Begleiter<sup>3</sup>, T. Reich<sup>2</sup>, H.J. Edenberg<sup>1</sup>, COGA collaborators.* 1) Indiana Univ Sch Medicine, Indianapolis, IN; 2) Washington University, St. Louis, MO; 3) SUNY Downstate Medical CenterBrooklyn, NY.

Polymorphisms in the alcohol metabolizing enzymes, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), have been consistently found to protect against alcoholism susceptibility in studies of East Asian populations. Linkage analyses in the primarily Caucasian, multiplex alcoholic families recruited in the Collaborative Study on the Genetics of Alcoholism (COGA) found evidence for a protective locus on chromosome 4, in the region containing the ADH gene cluster. To further evaluate the role of the 7 genes in the ADH gene cluster, 58 single nucleotide polymorphisms (SNPs) were genotyped in a sample of more than 2,200 individuals from alcoholic families. Analyses defining alcoholism based on the DSM-IV criteria detected strong evidence of association ( $p < 0.05$ ) using the pedigree disequilibrium test with eight of the nine SNPs in the ADH4 gene. The sliding window method, employed to form haplotypes using three adjacent SNPs, were all highly significant ( $p < 0.01$ ). These analyses suggest that the role of the ADH genes in affecting risk for alcoholism is not limited to the protective effects found in individuals of East Asian descent. This work was supported by the NIH grant U10AA08403 from the National Institute on Alcohol Abuse and Alcoholism (NIAAA). These analyses were also supported by AA13358 and AA00285.

**Joint analysis of chromosome 6p markers and antibodies to infectious agents in schizophrenia.** *J.J. Kim<sup>1</sup>, K.V. Chowdari<sup>1</sup>, J. Wood<sup>1</sup>, R. Yolken<sup>2</sup>, S. Bacanu<sup>1,3</sup>, B. Devlin<sup>1,3</sup>, V.L. Nimgaonkar<sup>1,3</sup>.* 1) Departments of Psychiatry, University of Pittsburgh, School of Medicine, WPIC, Pittsburgh, PA; 2) Stanley Laboratory of Developmental Neurovirology, Johns Hopkins School of Medicine, Baltimore, MD; 3) Departments of Human Genetics, University of Pittsburgh, School of Medicine and Graduate School of Public Health, WPIC, Pittsburgh, PA.

**OBJECTIVE:** Numerous studies have suggested linkage and associations of schizophrenia with polymorphisms localized on chromosome 6p, but they have been inconsistent. To resolve these inconsistencies, we have conducted multi-staged linkage and association analyses. We have also combined the genetic information with serological data relating to exposure to a range of infectious agents.

**METHODS:** Our sample included 100 affected sib-pairs, 158 case-parent trios and 161 unrelated controls. The cases were diagnosed as schizophrenia or schizoaffective disorder with DSM IV. Twenty two microsatellite markers, localized to a 21.5Mb region flanking the HLA region, were analyzed. We also assayed antibodies to cytomegalovirus (CMV), toxoplasma gondii, herpes simplex virus 1 and 2 (HSV-1 and HSV-2) among the patients and their parents.

**RESULTS:** TDT analysis revealed transmission distortion across the HLA region. These analyses attained nominal significance using the MCETDT program for D6S1663, D6S1545 and M6S230. A significant case-control difference, corrected for multiple comparisons, was detected at M6S125 using the CLUMP program. Significant transmission distortion was also observed at M6S125, when the sample was stratified on the basis of elevated titers of antibody to CMV among the patients and mothers.

**CONCLUSION AND FUTURE STUDIES:** Our analyses continue to provide evidence suggestive of linkage and association with schizophrenia in the HLA region. The results related to CMV exposure are intriguing, as they suggest a specific interaction between genetic variation and exposure to CMV, a putative pathogen for schizophrenia. We aim to analyze SNPs of candidate genes around the markers that showed significance in this study.

**Mutation screening of the apolipoprotein-L (APOL) genes and its association with schizophrenia.** *T. Ohtsuki<sup>1</sup>, T. Kojima<sup>2</sup>, YC. Shen<sup>3</sup>, YH. Han<sup>3</sup>, T. Arinami<sup>1</sup>.* 1) Department of Medical Genetics, University of Tsukuba, Tsukuba, Japan; 2) Department of Neuropsychiatry, Nihon University, Tokyo, Japan; 3) Institute of Mental Health, Beijing Medical University, Beijing, China.

Schizophrenia is a complex genetic disorder. However, the etiology of schizophrenia is poorly understood. Several genome-wide scans and meta-analysis of genome scans identified that 22q11-q13 is one of the susceptibility locus for schizophrenia. D22S683 on 22q12.3 showed significant association with schizophrenia in Japanese, European-American, and Chinese populations.

Recently, gene expression analysis by Mimmack et al (2002) identified that apolipoprotein - L1, L2, and L4 are significantly up-regulated in the prefrontal cortex of patients with schizophrenia. An apolipoprotein gene family (APOL1-6) is located near D22S683 on 22q12.3, and APOL1-4 are clustered as a result of tandem gene duplication and are highly homologous to each other.

In this study, we searched for mutations in all exons and exon-intron junctions of the APOL1-4 gene by direct sequencing in 48 Japanese patients with schizophrenia. We evaluated associations between schizophrenia and polymorphisms detected with a high frequency. We also evaluated associations in microsatellite markers located near the APOL gene. We performed a TDT (Transmission disequilibrium test) in 68 families with schizophrenia (16 Japanese families and 52 Chinese families). None of these polymorphic alleles were significantly associated with schizophrenia. However, two microsatellite markers were significantly associated with schizophrenia. In conclusion, we did not detect any evidence that the genomic variations in the APOL1-4 gene contribute to genetic susceptibility to schizophrenia in the Japanese and Chinese populations. However, we can not exclude the possibility that this genomic region is associated with expression of apolipoprotein-L genes in patients with schizophrenia.

Written informed consent was obtained from all subjects. This study was approved by Ethics Committee of each authors affiliation.

**Linkage and association studies of chromosome 16q in Italian inflammatory bowel disease families.** *M. Devoto*<sup>1</sup>, *V. Annese*<sup>2</sup>, *A. Latiano*<sup>2</sup>, *O. Palmieri*<sup>2</sup>, *P. Forabosco*<sup>3</sup>, *A. Ferraris*<sup>4</sup>, *H. Li*<sup>1</sup>, *M. Vecchi*<sup>5</sup>, *S. Ardizzone*<sup>6</sup>, *M. Cottone*<sup>7</sup>, *A. Andriulli*<sup>2</sup>, *B. Dallapiccola*<sup>4</sup>, *E. Rappaport*<sup>8</sup>, *P. Fortina*<sup>9</sup>. 1) Dept. Research, Nemours Children's Clinic, Wilmington, DE; 2) IRCCS-CSS, San Giovanni Rotondo, Italy; 3) CNR, Alghero, Italy; 4) CSS-Mendel and Universita La Sapienza, Roma, Italy; 5) IRCCS Osp. Maggiore, Milano, Italy; 6) Osp.Sacco, Milano, Italy; 7) Osp.Cervello, Palermo, Italy; 8) J. Stokes, Jr. Research Institute, Philadelphia, PA; 9) Dept.Medicine, Thomas Jefferson University, Philadelphia, PA.

Allelic variants of SNPs of the CARD15 gene, located on chromosome 16q, cause susceptibility to Crohn disease (CD), but not ulcerative colitis (UC). We previously found linkage to chromosome 16q in both CD and UC Italian families. In this study, we tested 90 Italian inflammatory bowel disease (IBD) families for the three most common CARD15 SNPs known to affect risk of CD (R702W, G908R and L1007fsinsC) and compared their frequencies in probands against 108 normal controls. Allele and phenotype frequencies at all three SNPs were significantly different between CD probands and controls, but not between UC probands and controls. When all three SNPs were combined into a single CARD15 phenotype, the relative risk of CD for carriers of two variants (either homozygotes or compound heterozygotes) compared to non-carriers was highly significant (OR=41.41, 95% CI=(4.86, 352.95), p=0.00001) whereas the relative risk for heterozygotes at only one SNP was not (OR=1.63, 95% CI=(0.57, 4.69), p=0.39). After removing 26 CARD15 positive families, we performed a GENEHUNTER model-free linkage analysis with 18 microsatellites spanning 28 cM around CARD15. In the CD group, there was no significant evidence of linkage after the CARD15 positive families were removed (all p-values > 0.05). In contrast, significant linkage to D16S408, located 12 cM away from CARD15, was found in 37 CARD15 negative UC families (p = 0.003). Our results suggest that additional genes in the pericentromeric region of chromosome 16 may affect risk of inflammatory bowel disease. We are currently testing SNPs in candidate genes near D16S408 for association to IBD in our families' probands.

**Three novel SCN1A missense mutations in generalized epilepsy with febrile seizures plus.** G. Annesi<sup>1</sup>, S. Carrideo<sup>1</sup>, G. Incorpora<sup>3</sup>, D. Civitelli<sup>1</sup>, A. Polizzi<sup>3</sup>, F. Annesi<sup>1</sup>, P. Tarantino<sup>1</sup>, I.C. Cirò Candiano<sup>1</sup>, P. Spadafora<sup>1</sup>, E.V. De Marco<sup>1</sup>, U. Aguglia<sup>4</sup>, A. Labate<sup>1</sup>, L. Pavone<sup>3</sup>, A. Quattrone<sup>1,2</sup>, A. Gambardella<sup>1,2</sup>. 1) Inst Neurological Sci, National Research Council, Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia Catanzaro, Italy; 3) Institute of Pediatrics, University of Catania, Italy; 4) Regional Epilepsy Centre, Hospital of Reggio Calabria, Italy.

**Summary.** Purpose: to screen 13 Italian families with generalized epilepsy with febrile seizures plus (GEFS+) for mutations in SCN1A, SCN1B and GABRG2 genes. Patients and methods. We selected 13 unrelated families with GEFS+. There were 37 affected members, and most of them had febrile seizures (FS) or FS plus. Genomic DNA was extracted by standard methods. The 26 exons of SCN1A, five exons of SCN1B and nine exons of GABRG2 were individually amplified using primers based on an intronic sequence. PCR products were analyzed by single strand conformation polymorphism and sequence. Results. In three GEFS+ families, we identified three novel missense mutations of the SCN1A gene, which co-segregated with the disorder and never occurred in 100 unrelated controls. The first Tyr779Cys mutation was located in the DII/S1 segment, the second Met1841Thr and the third Ile1944Thr were located within the intracellular C terminal region. The latter mutation was associated with severe myoclonic epilepsy at infancy (SMEI). There were no variants of SCN1B or GABRG2 genes that co-segregated with the GEFS+ phenotype. Conclusions. The results of our study further support the role of the SCN1A gene in the etiology of GEFS+. Our findings also reinforce the belief that SMEI represents the very severe end of the spectrum within GEFS+ phenotype. Reference Annesi G., Gambardella A. et al. (2003). Two novel SCN1A missense mutations in Generalized Epilepsy with febrile seizures plus. *Epilepsia* (in press).

**A possible role of gamma-aminobutyric acid A receptor-associated protein (GABARAP) in nicotine dependence.**

*J. Beuten*<sup>1</sup>, *J. Ma*<sup>1</sup>, *R. Dupont*<sup>2</sup>, *K. Crews*<sup>3</sup>, *T. Payne*<sup>3</sup>, *M. Li*<sup>1</sup>. 1) Department of Psychiatry, University of Texas Health Science Center, San Antonio, TX; 2) Departments of Psychiatry and Allied Health, The University of Tennessee Health Science Center, Memphis, TN; 3) Departments of Diagnostic Sciences and Medicine, The University of Mississippi Medical Center, Jackson, MS.

Nicotine dependence is a complex trait with strong genetic and environmental influences. Twin and adoption studies have demonstrated that the heritability for smoking initiation and smoking persistence is at least 50%. Based on the results from our genome-wide linkage analysis on the Framingham Heart study population, 13 genomic loci located on 12 different chromosomes are likely to harbor susceptibility genes for smoking behavior. During the past several years, we have been collecting phenotypic information and blood samples from both smoker and non-smoker members of predominantly Caucasian and African-American families. Currently more than 1,400 subjects have been recruited and their DNA samples are available for genetic analysis. To determine possible association between candidate genes and nicotine dependence, we genotyped these DNA samples by the TaqMan assay with SNPs selected from candidate genes located within the linked regions, and known to play a role in drug abuse and/or dopamine metabolism pathways. One of the genes of interest in this study is gamma-aminobutyric acid A receptor-associated protein (GABARAP), which promotes clustering of the GABA(A) neurotransmitter receptors and is located on chromosome 17p13.2. Association analysis using the ASSOC program of S.A.G.E. indicated that a significant association exists between the G/T polymorphism at amino acid position 164 in the GABARAP gene and nicotine dependence in the Caucasian population under both the additive and dominance genetic model ( $P < 0.04$ ). In the African-American population, however, no significant association between this G/T polymorphism of the GABARAP gene and nicotine dependence was found. These findings support a possible role of GABARAP in nicotine dependence in the Caucasian population (Supported by DA-12844).

**A mixed epigenetic-genetic model for autism with the ubiquitin ligase *UBE3A* as a candidate principal gene.** *T. Sahoo*<sup>1</sup>, *Y. Jiang*<sup>1</sup>, *M. Shinawi*<sup>1</sup>, *R.C. Michaelis*<sup>2</sup>, *I. Buyse*<sup>1</sup>, *C.D. Kashork*<sup>1</sup>, *R.J. Schroer*<sup>2</sup>, *B.B. Roa*<sup>1</sup>, *D.W. Stockton*<sup>1</sup>, *R.S. Spielman*<sup>3</sup>, *R.E. Stevenson*<sup>2</sup>, *L.G. Shaffer*<sup>1</sup>, *A.L. Beaudet*<sup>1</sup>. 1) Human & Molec Gen, Baylor Col Medicine, Houston, TX; 2) Greenwood Genetic Center, S. Carolina; 3) Univ. of Pennsylvania, Philadelphia, Pennsylvania.

We propose a mixed epigenetic-genetic model for autism with a principal imprinted gene in chromosome 15q. The Angelman gene (E6-AP ubiquitin-protein ligase; *UBE3A*) maps in this region, is imprinted with paternal silencing in brain, and affects neurological function and learning. We hypothesize that over-expression of an imprinted gene in 15q11-q13 is the major cause of autism, and *UBE3A* is the strongest candidate with brain-specific failure of paternal silencing as a major mechanism. Using a subset of AGRE and NIMH autism sib-pairs a small number of families with maternal duplications of 15q11-q13 were identified. Genotyping of affected sib pairs showed increased sharing of paternal alleles in 15q11-q13, with the greatest sharing centromeric to the imprinting center at D15S817 with a  $X^2$  of 5.48 ( $P=0.02$ ). We propose that autism may be caused in the majority of cases by imprinting defects arising on normal or susceptible paternal chromosome 15 during spermatogenesis or after fertilization prior to MZ twinning, leading to brain-specific over-expression of *UBE3A*. We are testing the hypothesis that autism etiology involves a gene-nutrient interaction analogous to but opposite from the finding in neural tube defects (NTDs), such that increased folate intake for the maternal-fetal unit will increase rather than decrease risk for autism; in addition the hypomorphic allele for MTHFR would be protective and be under-represented in autism. Testing for the frequency of MTHFR genotypes (222A->V) shows a lower frequency of V/V allele in autistic cases compared to controls but the difference may not be statistically significant. A transmission disequilibrium test (TDT) to test for parent-of-origin bias in the transmission of the hypomorphic allele revealed a statistically significant increase in paternal transmission of the A allele to autistic offsprings ( $X^2=4.403$ ;  $p<0.05$ ) suggesting that V would be a protective allele.

**Case-control, TDT and gene-gene interaction analysis of MTHFR, BCL3 and TGF $\alpha$  genes in nonsyndromic clefting Brazilian families.** *D.A. Gaspar<sup>1,2</sup>, S.R. Matioli<sup>2</sup>, R.C. Pavanello<sup>1,2</sup>, B.C. Araújo<sup>3</sup>, M. André<sup>4</sup>, S. Steman<sup>5</sup>, N. Alonso<sup>5</sup>, M.R. Passos-Bueno<sup>1,2</sup>.* 1) CEGH, IBUSP, Univ de São Paulo; 2) Depto Biologia, IB-USP, Univ de São Paulo; 3) Hospital Menino Jesus; 4) FOU SP, Univ de São Paulo; 5) Depto de Cirurgia Plástica, FMUSP; São Paulo, Brazil.

Nonsyndromic cleft lip with or without cleft palate (CL/P), one of the most common birth defects in humans, is a complex disorder in which both genetic and environmental factors influence risk. The 677 CT polymorphism at the MTHFR gene has been associated with CL/P in some populations and some authors, including a previous report by our own group, found that the maternal MTHFR genotype might also be a risk factor for CL/P to their descendants. However, this issue is still controversial. In order to elucidate the role of the 677 CT/MTHFR polymorphism to the predisposition to CL/P, we performed a case-control study of this functional SNP in a large sample of individuals ascertained in two different regions of Brazil (São Paulo and Ceará): 424 CL/P patients, 336 mothers of CL/P patients and 634 controls matched by ethnic and social background to patients. TDT was also carried out in 105 CL/P patients and their parents. This polymorphic system is in Hardy-Weinberg equilibrium in all subgroups ( $P > 0.05$ ). We also observed that T allele and the TT genotype frequencies did not differ among CL/P patients (9% and 30%), mothers of CL/P patients (9% and 29%) and controls (8% and 29%) ( $P > 0.05$ ). The lack of association between the 677T allele and CL/P was further confirmed through the TDT strategy ( $\chi^2 = 3.7468$ , 1df;  $P = 0.05$ ). We have also evaluated a possible interaction between the 677 CT/MTHFR polymorphism and the at risk-alleles for CL/P at BCL3 (allele 135bp;  $n = 85$ ) or TGF $\alpha$  (allele C2/TaqI;  $n = 162$ ) genes. We did not observe any indication of gene-gene interaction between MTHFR and either BCL3 or TGF $\alpha$ . In conclusion, maternal or patients genotypes harboring the 677T/MTHFR allele do not increase the risk of CL/P; in addition, this locus does not seem to interact with TGF $\alpha$  and BCL3 in the susceptibility to this malformation, although the sample sizes available for the various genotypic combinations may limit statistic power.

**A Study of DR-DQ Matched T1D Cases and Controls in a Continental Italian Population Reveals Susceptibility Effects of DPB1 and HLA-A Alleles.** *J.A. Noble<sup>1</sup>, A.M. Valdes<sup>2</sup>, Q. McGrue<sup>1</sup>, A. Galgani<sup>2</sup>, A. Petrone<sup>2</sup>, S. Di Cola<sup>2</sup>, H.A. Erlich<sup>3</sup>, P. Pozzilli<sup>2</sup>, R. Buzzetti<sup>2</sup>.* 1) Childrens Hosp Oakland Res Ins, Oakland, CA; 2) University of Rome, Rome, Italy; 3) Roche Molecular Systems, Alameda, CA.

T1D patients from the Lazio region of central Italy with HLA DR-DQ genotypes classified as high-risk (DR3/DR4, DR3/DR3, and DR4/DR4) were compared to controls with the same set of genotypes in an effort to identify risk factors for T1D in addition to the HLA DR- and DQ-encoding loci. The HLA class II locus DPB1 and the HLA class I locus A were genotyped and analyzed in an initial set of 42 patients and 147 controls. Consistent with previous reports, the frequencies of DPB1\*0301, DPB1\*0202, and A\*2402 were higher in patients than in controls, while frequencies of DPB1\*0402 and A\*0101 were lower in patients than in controls, although these differences did not reach statistical significance in the combined data. However, analyses of the subset of DR3/DR3 patients and controls revealed statistically significant differences in some allele frequencies. Significantly more DR3/DR3 patients had at least one copy of the DPB1\*0301 allele than did the DR3/DR3 controls ( $p = 0.035$ ), consistent with several previous reports of a predisposing effect for this allele. For HLA-A, analysis of only the DR3/DR3 patients and controls revealed a significant excess of A\*0301 in patients ( $p = 0.021$ ). This possible predisposing effect of A\*0301 has not been previously observed. A\*0301 is in strong linkage disequilibrium with the highly protective DR2 haplotype (DRB1\*1501-DQB1\*0602), such that the allele appears somewhat protective in other studies not matched for DR and DQ-encoding loci. Results from examining the allele in the context of DR-DQ-matched samples may be more representative of its true effect on T1D susceptibility. Collection and genotyping of additional samples is in progress. The larger sample set will be utilized not only to increase the power of the DPB1 and HLA-A analyses but also to examine other candidate T1D susceptibility loci in this DR-DQ matched case-control study.

**Epistasis in Polygenic Disorders: The Effect of Age of the Mother at the Birth of her First Child.** *D.E. Comings*<sup>1</sup>, *J.P. MacMurray*<sup>2</sup>. 1) Medical Genetics, City of Hope Medical Center, Duarte, CA; 2) Carlsbad Science Foundation, Monrovia, CA.

**Purpose:** Epistasis refers to a situation in which the effect of one unit is not predictable unless the value of the other unit is known. This includes gene gene interactions and gene non-gene interactions.<sup>1</sup> We previously identified maternal age as an epistatic factor for the interaction of a LEP gene microsatellite polymorphism with age of onset of menarche.<sup>2</sup> When the maternal age was 25 years of age, an early age of menarche was associated with the LEP short alleles. By contrast, when the maternal age was 26 years, an early age of menarche was associated with the LEP long alleles. The purpose of this study was to investigate two questions: a) was maternal age an epistatic factor for other gene trait interactions? and b) was maternal age or maternal age at the birth of the first child (Maternal age 1st) more important?

**Methods:** We examined the association of the DRD1 gene (Dde I polymorphism) with four phenotypes in a database of Tourette syndrome probands: OCD, stuttering, conduct disorder and ADHD. **Summary:** Both maternal age and maternal age 1st born were epistatic factors for the interaction of the DRD1 gene with OCD and stuttering. Maternal age 1st born was consistently a stronger epistatic factor than maternal age. This may reflect a different genetic background in individuals who have children early versus those who have them late. We observed a special type of epistasis in which the association of the 11 and 22 homozygotes was in opposite direction for the two epistatic conditions. We have termed this *genostasis* (geno = genotype, stasis = reversal). Since the two effects neutralize each other, significant gene trait associations could be missed if genostatic factors were not included in the analysis. Maternal age 1st may prove to be an important genostatic factor for many other gene-trait interactions.

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**Search for association with *Klotho* allele variation and atherosclerosis in the Baltimore Longitudinal Study on Aging.** A. Bektas, D. Taub, S. Najjar, D. Muller, L. Ferrucci, C.A. Francomano. National Institute on Aging/NIH, Baltimore, MD.

Numerous studies on the *klotho* mutant mouse have demonstrated that the *klotho* gene is involved in the suppression of several aging phenotypes, including atherosclerosis. Arking et al (2002, 2003) showed that the KL-VS allele of the *klotho* gene might be as a functional variant that influences longevity and an independent risk factor for coronary artery disease (CAD). We looked for an association between the KL-VS allele and the phenotype of atherosclerosis in the Baltimore Longitudinal Study on Aging (BLSA) population. Our initial analysis included Caucasian participants, ages (66) to (92) with and without atherosclerotic disease (coronary artery disease and/or stroke). We screened a healthy control group for the previously described KL-VS allele, containing six sequence variants in complete linkage disequilibrium. The presence of the KL-FC and KL-VS alleles of the *klotho* gene were determined by sequencing healthy control subjects (N=148). In patients with coronary artery disease-atherosclerosis-stroke (CSAD) (N=83) the alleles were detected by restriction fragment length polymorphism (RFLP) analysis. We found that the frequency of KL-FC allele was (0.83) in the healthy control group and (0.86) in CSAD group ( $P>.05$ ). The frequency of KL-VS allele was (0.17) in healthy control group and (0.14) in CSAD group ( $P>.05$ ). Therefore, these data did not support an association between KL-VS allele of *klotho* gene and CSAD disease in BLSA white population. Inclusion of additional black healthy control subjects and CSAD patients resulted in allele frequencies of 0.84 for the KL-FC allele in the healthy control group, and 0.84 in the CSAD group ( $P>.05$ ). The KL-VS allele frequency was 0.16 in the healthy control group, and 0.16 in the CSAD group ( $P>.05$ ). Thus, the data do not support an association between atherosclerotic disease and the *klotho* KL-VS allele in the BLSA population. Further work in additional populations and with age stratification is required.

Program Nr: 2077 from 2003 ASHG Annual Meeting

**Investigation of the CHRNA4 gene for a possible role in tobacco use and addiction.** *M.A. Ehringer, S. Young, R. Corley, M. Stallings, A.C. Collins, J.K. Hewitt.* Institute Behavioral Genetics, Univ Colorado, Boulder, CO.

That genetic factors contribute to tobacco use and nicotine dependence has been well established through adoption, twin, and family studies. However, the specific genes contributing to an individual's risk of becoming addicted to nicotine remain unknown. Recent studies using mouse models have identified a polymorphism in the gene for the 4 subunit of the neuronal nicotinic receptor (Chrna4) which is linked to increased sensitivity to nicotine. This project involves utilizing the genomics resources developed through the human genome project to examine SNPs in the human CHRNA4 gene. Genotyping assays for six selected SNPs have been developed using a modification of the ABI PRISM SNaPshot ddNTP Primer Extension Kit. 333 late adolescents/young adults aged 17 to 21, representing a Colorado community sample, have been previously assessed for tobacco-related phenotypes. Genotypes for the six CHRNA4 SNPs are being determined for these individuals. Preliminary data indicate that five of the six SNPs are present with minor allele frequencies between 0.04 and 0.38; however, one of the SNPs is not polymorphic in this sample. Calculations to determine the D measure of linkage disequilibrium suggest that D between the SNPs varies from 0.11 to 0.77. Upon completion of genotyping of all SNPs for all subjects, we will test for a possible association between the variants and two tobacco-related phenotypes. Evaluating a quantitative measure of typical pattern of use, we will use linear regression to test for a possible association between frequency of use and genotype. Similarly, approximately half of the subjects display at least one DSM-IV dependence symptom for nicotine, while the other half do not. A chi-square test will be used to test whether a particular genotype for one or more of the SNPs is associated with dependence symptoms. The identification of particular SNPs that may be associated with tobacco use and addiction should facilitate greater understanding of the underlying genetic and biological mechanisms contributing to this disease. This work has been supported by the Colorado Tobacco Research Program (IDEA grant #21-034) and NIH/NIDA P60 DA 11015-05.

**MECP2 variants in psychiatric diseases: possible association with autism.** *J. Feng<sup>1</sup>, A. Shibayama<sup>1</sup>, C. Glanzmann<sup>1</sup>, J. Yan<sup>1</sup>, E. Cook Jr.<sup>2</sup>, N. Craddock<sup>3</sup>, I. Jones<sup>3</sup>, D. Goldman<sup>4</sup>, L. Heston<sup>5</sup>, S. Sommer<sup>1</sup>.* 1) Dept Molecular Genetics, City Hope Natl Medical Ctr, Duarte, CA; 2) University of Chicago, Dept. of Psychiatry, Chicago, IL; 3) University of Birmingham, Division of Neuroscience, Queen Elizabeth Psychiatric Hospital, Birmingham, UK; 4) NIAAA, NIH, Dept. of Psychiatry, Bethesda, MD; 5) University of Washington, Dept. of Psychiatry, Seattle, WA.

Mutations in the gene coding methyl-CpG-binding protein 2 (MECP2) cause Rett syndrome (RTT) and have also been reported in a number of X-linked mental retardation syndromes. Putative mutations have recently been described in a few autistic patients and a boy with language disorder and schizophrenia. In this study, DNA samples from individuals with schizophrenia and other psychiatric diseases were scanned in order to explore whether phenotypic spectrum of mutations in the MECP2 gene extends beyond the traditional diagnosis of RTT and X-linked mental retardation syndromes. The coding regions, adjacent splicing junctions and highly conserved segments of the 3'-untranslated region (3'-UTR) were examined in 214 patients including 106 with schizophrenia, 24 with autism and 84 patients with other psychiatric diseases by DOVAM-S. To our knowledge, this is the first analysis of variants in highly conserved regions of the 3' UTR of this gene. A total of 1.5 megabases was scanned (5.2 kb per haploid gene). Higher frequencies of missense and 3-UTR variants were found in autism. One missense and two 3-UTR variants were found in 24 patients with autism versus one patient with a missense change and one patient with a 3-UTR variant in 144 ethnically similar patients without autism ( $p=0.02$ ). These mutations suggest a possible association between the MECP2 gene and autism, warranting further study.

**Mutation analysis of *CYP11B1* and *CYP11B2* in patients with increased 18-hydroxycortisol production.** *J. Nicod, B. Dick, F.J. Frey, P. Ferrari.* Division of Nephrology and Hypertension, University Hospital, Berne, Switzerland.

In patients with glucocorticoid remediable aldosteronism (GRA), a rare hypertensive disorder caused by the presence of a chimeric aldosterone synthase (*CYP11B2*) and 11-hydroxylase (*CYP11B1*) gene, high level of urinary 18-hydroxycortisol (18OHF) excretion are observed. In some patients with hypertension increased urinary 18OHF secretion is also found in the absence of the hybrid *CYP11B1/CYP11B2* gene. We hypothesised that gene variants of *CYP11B1* or *CYP11B2* may be linked to this abnormal glucocorticoid production.

The urinary steroid profile was analysed by gas chromatography/mass spectrometry in 429 hypertensive patients and 98 (23%) thereof tested positive for increased 18OHF excretion. After correction for total cortisol excretion, 12 subjects showed an abnormally high 18OHF excretion. For genotyping DNA was obtained from 6 of these patients. All tested negative for the hybrid *CYP11B1/CYP11B2* gene and were further analysed for mutations in all exons and promoter regions of both *CYP11B1* and *CYP11B2* by single strand conformation polymorphism (SSCP) and sequencing when appropriate.

The genetic analysis of the two genes revealed the presence of 9 variants in *CYP11B2* and 3 in *CYP11B1*. In addition to published polymorphic sites, we identified 2 new variants in *CYP11B2* but no new variants in *CYP11B1*. The newly identified *CYP11B2* mutations are a C/T single nucleotide exchange located in the first intron and a double nucleotide exchange at the 5 splice site of exon 8. The mutated sequence corresponds to the sequence of *CYP11B1* indicating a gene conversion. This suggests that the mutant is not likely to affect splicing. Thus, none of the genetic variants identified explains the high urinary excretion of 18OHF.

We present here a complete method for the genetic analysis of the *CYP11B1* and *CYP11B2* genes. By this method we could not identify genetic variants responsible for a GRA-like phenotype. The presence of high levels of 18OHF should not be used alone as a diagnosis tool for GRA.

***FOXC2* variation in breast cancer survivors with secondary lymphedema.** *R.L. Miller<sup>1</sup>, E. Feingold<sup>1</sup>, K. Lorenzo<sup>1</sup>, K.L. Levinson<sup>1</sup>, J.W. Dunlap<sup>1</sup>, M.A. Kimak<sup>1</sup>, E.C. Lawrence<sup>1</sup>, A. Soran<sup>4</sup>, V.G. Vogel<sup>2</sup>, J.H. Esman<sup>3</sup>, R.E. Ferrell<sup>1</sup>, D.N. Finegold<sup>5</sup>*. 1) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Magee-Womens Hospital and UPCI, Pittsburgh, PA; 3) Div. of Physical Medicine and Rehabilitation, UPMC-S, Pittsburgh, PA; 4) NSABP Center, Pittsburgh, PA; 5) Dept Pediatrics, Children's Hospital of Pittsburgh, Pittsburgh, PA.

Secondary lymphedema following treatment of breast cancer develops in 15-20% of breast cancer survivors (Rockson, 2001). There are no well defined differences between surgical technique, grade of tumor, or environmental risk factors that accurately predict who is at the highest risk for developing secondary lymphedema. We hypothesized that genetic variation may predispose a woman to a greater risk for developing secondary lymphedema following breast cancer surgery. Hereditary primary lymphedema is caused by at least two genes, forkhead box C-2 (*FOXC2*) and vascular endothelial growth-factor receptor-3 (*VEGFR3*), which when mutated, alter the development and function of the lymphatic system. We performed detailed sequence analysis of *VEGFR3* and *FOXC2* for 45 cases with secondary lymphedema and 48 controls without secondary lymphedema in our study population of breast cancer survivors. A single non-conservative amino acid substitution, G460E, in *FOXC2* was identified in 2 cases, and no controls. This variation did not occur in 282 alleles genotyped in an unselected mixed Caucasian population. This substitution, outside of the fork-head domain, may provide an increased risk for secondary lymphedema when combined with environmental factors.

**The Dopamine-4 Receptor Gene Associated with Increased Body Mass: A Behavioural Model of Obesity in Two Female Psychiatric Populations.** *R.D. Levitan<sup>1</sup>, M. Masellis<sup>2</sup>, A.S. Kaplan<sup>3</sup>, R.W. Lam<sup>4</sup>, V.S. Basile<sup>2</sup>, C. Davis<sup>3</sup>, P. Muglia<sup>2</sup>, S. Tharlamangam<sup>2</sup>, S.H. Kennedy<sup>5</sup>, J.L. Kennedy<sup>2</sup>.* 1) CAMH, University of Toronto, Department of Psychiatry; 2) Neurogenetics Section, CAMH, University of Toronto, Department of Psychiatry; 3) Eating Disorders Program, University Health Network, University of Toronto; 4) University of British Columbia, Department of Psychiatry; 5) University Health Network, University of Toronto, Department of Psychiatry.

**Background:** There is growing interest in delineating behavioural risk factors for increased body mass. Brain dopamine activity mediates the rewarding properties of food and plays a role in obesity. In the current study we examined whether variation in the dopamine-4 receptor gene (DRD4) was associated with maximal lifetime body mass and obesity in two female psychiatric conditions characterized by overeating. **Methods:** The study sample consisted of 132 women with winter seasonal affective disorder who reported increased intake of high carbohydrate/high fat foods when depressed, and 115 women with binge eating and purging in the context of bulimia nervosa. Maximal lifetime body mass and rates of obesity were compared across the two genotypic groups defined by the presence or absence of the 7-repeat allele of the DRD4 exon III VNTR polymorphism. **Results:** In the overall sample, there was a significant association between the 7-repeat allele and both continuous and categorical measures of increased body mass. The lifetime rates of obesity for individuals with and without the 7-repeat allele were 28.2 % and 13.6 % respectively ( $p=0.0047$ ). This pattern held true in both the SAD group and in the BN group considered separately, with the smaller group of non-depressed bulimics showing a particularly marked effect. Further analysis revealed an association between the 7-repeat allele and binge eating in the SAD group, further supporting a link between DRD4 and increased eating behaviour per se. **Interpretation:** Pending replication in other samples, these results point to a genetic vulnerability factor that could help in the early identification and treatment of females at higher risk for obesity due to pathologically increased eating behaviour.

**TGF1 - SNP Discovery and Evaluation as a Candidate Gene for Asthma in Puerto Rican and Mexican**

**Asthmatics.** *D.L. Lind<sup>1</sup>, S. Choudhry<sup>2</sup>, A. Reinhart<sup>1</sup>, E. Ziv<sup>3</sup>, P.C. Avila<sup>3</sup>, S. Nazario<sup>4</sup>, J. Casal<sup>4</sup>, A. Torres<sup>4</sup>, J.R. Rodriguez-Santana<sup>5</sup>, J. Salas<sup>6</sup>, M. Selman<sup>6</sup>, R. Chapela<sup>6</sup>, W. Rodriguez-Cintron<sup>4</sup>, S.T. Weiss<sup>7</sup>, E.K. Silverman<sup>7</sup>, J.G. Ford<sup>8</sup>, D. Sheppard<sup>2,3</sup>, P.-Y. Kwok<sup>1</sup>, E.G. Burchard<sup>2,3</sup>.* 1) Cardiovascular Research Inst., UCSF, San Francisco, CA; 2) Lung Biology Center, SFGH, San Francisco, CA; 3) Dept of Medicine, UCSF, San Francisco, CA; 4) San Juan VAMC, Univ of Puerto Rico School of Medicine, San Juan, PR; 5) Pediatric Pulmonary Program of Puerto Rico, San Juan, PR; 6) INER, Mexico City, MX; 7) Brigham and Women's Hospital, Boston, MA; 8) The Harlem Lung Center, Harlem Hospital and Columbia Univ, New York, NY.

Transforming growth factor-1 is a multifunctional cytokine that acts as a growth inhibitor in many cell types and also mediates inflammatory responses. We began our investigation of TGF1 as a candidate gene for asthma by performing SNP discovery in 72 asthmatic individuals (24 Mexicans, Puerto Ricans and African Americans) as well as in 120 normal individuals (40 African Americans, Asians and Caucasians). We sequenced the exons and 300 bp flanking each exon as well as 400 bp upstream of the transcription start, covering a total of 4.4 kb. We found 19 SNPs in this region, 5 of which had allele frequencies 5% in the asthmatic or normal individuals. 4 of the 19 SNPs caused amino acid changes, and 2 of these were present at 5% allele frequency in both asthmatics and at least one population of normal individuals. The common SNPs and coding SNPs will be genotyped, making use of the haplotype structure to limit redundancy. The study population will be 583 Puerto Rican and Mexican trios (asthmatic proband and both biological parents) from the Genetics of Asthma in Latino Americans (GALA) study. We chose to study these two populations because in the U.S., Puerto Ricans have the highest asthma prevalence, morbidity and mortality, and Mexicans have the lowest. Genotype data from the trios will allow us to perform TDT analyses. We also have 400 matched control samples, which will allow us to perform case-control analyses. These results will allow us to evaluate TGF1 as a candidate gene for asthma in Puerto Ricans and Mexicans.

**Association Analysis of Genetic Polymorphisms in Sac, Pank4, Casp9, and Cdc212 genes with Type 2 Diabetes in Han People of Northern China.** G. Wu<sup>1</sup>, J. Zhao<sup>2</sup>, C. Yang<sup>1</sup>, H. Wang<sup>4</sup>, J. Zuo<sup>1</sup>, Y. Wang<sup>3</sup>, Z. Liu<sup>1</sup>, Y. Zhang<sup>1</sup>, Y. Shen<sup>5</sup>, B. Qiang<sup>5</sup>, Z. Yao<sup>5</sup>, W. Huang<sup>3</sup>, C. Zhu<sup>3</sup>, F. Fang<sup>1</sup>. 1) National Laboratory of Medical, Institute of Basic Medical Sci, Beijing; 2) Human Genetics Ctr, Univ Texas, Houston; 3) Chinese National Human Genome Center at Shanghai, Shanghai; 4) Peking Union Medical College Hospital, Beijing; 5) Chinese National Human Genome Center at Beijing, Beijing, China.

In our previous genome-wide screen, we have localized the susceptible loci to chromosome 1. 3 regions: 1p36.23~1p36.33, 1q24.3~25.1 and 1q42.12~42.13, were showed evidence of linkage with type 2 diabetes. Sequencing was applied to detect single nucleotide polymorphisms (SNPs) on the promoters and complete coding regions of 34 candidate genes within above three regions in Chinese population, and a case-control study was performed to explore these SNPs correlations with type 2 diabetes mellitus in Han people of Northern China. The 34 genes chosen were sequenced to detect SNPs in 11 cases and 8 controls of Chinese population. Among the detected SNPs, 124 SNPs were chosen to perform case-control study in 147 normal Han people of Northern China and 222 type 2 diabetes patients. 4 SNPs in SAC (Soluble Adenylyl Cyclase) gene were showed significantly statistical difference. One genetic variation (GGT~GGC)(p=0.003) occurred at the 385th amino acid. It does not change the amino acid. But the variant is 15 nucleotides away from the splicing sites of exon11 that is alternatively spliced. SAC represents a novel mammalian adenylyl cyclase structurally, molecularly, and biochemically distinct from the G protein-regulated, transmembrane adenylyl cyclases (tmACs). The sac protein is an evolutionarily conserved bicarbonate sensor. A new mutation in gene Pank4 (Pantothenate Kinase 4) changed the 547th amino acid (V547A), and showed significantly statistical difference (P=0.028). Pank4 gene encodes a key regulatory enzyme in the biosynthesis of coenzyme A (CoA) in mammalian cells. The mutation maybe changes the function of the protein directly. Also SNPs in the introns in gene Casp9 and Cdc212 showed association (P=0.041,P=0.043) between these SNPs and type 2 diabetes.

**Alcohol Dehydrogenase 4 polymorphisms as a risk factor for alcoholism in Brazilian patients.** *M. Zatz<sup>1</sup>, C. Guindalini<sup>1,3</sup>, S. Scivoletto<sup>2</sup>, R.G.M. Ferreira<sup>1</sup>, G. Breen<sup>3</sup>, M. Zilberman<sup>2</sup>, M.A. Peluso<sup>2</sup>, E. Quedas<sup>1</sup>, A. Nishimura<sup>1</sup>.* 1) Human Genome Research Center, Department of Biology, Institute of Biosciences - University of Sao Paulo; 2) GREA Interdisciplinary Group of Studies on Alcohol and Drugs - Institute and Department of Psychiatry, Medicine Faculty - University of Sao Paulo; 3) Section of Genetics, Division of Psychological Medicine, Institute of Psychiatry-Kings College London.

There is much evidence that the variability in alcohol absorption, distribution and metabolism is a result of both genetic and environmental factors. To date the genes with the strongest associations with alcoholism are those that encode the enzymes involved in alcohol metabolism, the alcohol dehydrogenase (ADH2 and ADH3) and the aldehyde dehydrogenase (ALDH2). The aim of this study was to test the influence of three polymorphisms found in the promoter region of the ADH4 gene, comparing a group of Brazilian alcohol dependent patients (n=92) and normal controls (n=92). We found that the allele C at -75bp position and the allele A at -159bp position were significantly over-represented in the patient group. The haplotype analyze showed that the combination of both risk alleles increases the risk of developing alcoholism by 3 times (OR=2.9, 95%CI: 1.47-5.89). These results suggest that ADH4 may play a role in the etiology of alcoholism, since the association is functionally plausible and has a large effect size, although these promising results require further confirmation. FAPESP/CEPID and CNPq supported this work.

**IL1RN variants are associated also in the general population with asthma and IgE levels.** *M. Wjst<sup>1</sup>, H. Gohlke<sup>1</sup>, N. Herbon<sup>1</sup>, C. Braig<sup>1</sup>, M. Bahnweg<sup>1</sup>, J. Heinrich<sup>1</sup>, K. Richter<sup>2</sup>, J. Altmueller<sup>3</sup>, G. Malerba<sup>4</sup>, P. Pignatti<sup>4</sup>.* 1) Institute of Epidemiology, GSF National Research Center for Environment and Health, Munich-Neuherberg, Germany; 2) Pulmonary Research Institute at Grosshansdorf Hospital, Center for Pneumology and Thoracic Surgery, Grosshansdorf, Germany; 3) Genome Mapping Center, Max-Dellbrueck-Zentrum, Berlin-Buch, Germany; 4) Department of Mother and Child, Biology and Genetics, University of Verona, Italy.

Following a genomewide screen in German/Swedish affected sib pair families we previously found linkage with asthma at the 400 kB interleukin-1 gene cluster on chromosome 2q12-2q14. A further screening of more than 150 tightly-spaced SNPs showed significant association on a restricted LD area around the interleukin 1 receptor antagonist IL1RN which could be confirmed in an Italian family study. IL1RN encodes IL-1r, an anti-inflammatory cytokine. We have now further tested 13 SNPs in IL1RN (2234678, 878972, 315936, 392503, 439154, 442710, 447713, 128964, 434792, 454078, 440286, 3087271, 895496) in a population based survey of 590 adult individuals who were participating in the European Community Respiratory Health Survey ECRHS II. 9% had asthma, 18% were bronchial hyperreactive after methacholine provocation and 26% had IgE levels greater than 100 kU/l. 2 of 13 SNPs were also here significantly associated with asthma (315936,  $P=0.024$ ; 454078  $P=0.036$ ). Haplotype-trait associations were even stronger (A-A-A-C-T-C-A-G-G-A-A-A-G,  $P=0.002$ ; A-A-A-C-T-C-G-G-G-A-A-A-A,  $P=0.004$ , G-G-C-T-C-G-A-T-A-G-T-C-G,  $P<.0001$ ). 7 of the tested 13 SNPs showed an association with total IgE levels (again 315936 with  $P=0.016$  and 454078 with  $P=0.006$ ) while BHR was not associated with any SNP. Resequencing of the two IL1RN promoters, association studies with IL-1r serum level, as well as functional studies are underway. We speculate that one of the IL1RN associated SNPs antagonizes the environmental induced IL1-r production leading to inflammation and sensitization of the bronchial system.

**Searching for working memory genes: preliminary data on COMT genotypes.** *H. Xu<sup>1, 2</sup>, G. Bruder<sup>3</sup>, J. Keilp<sup>3</sup>, M. Shihkman<sup>3</sup>, E. Schori<sup>3</sup>, J. Gorman<sup>4</sup>, T.C. Gilliam<sup>1, 2</sup>.* 1) Department of Genetics and Development, Columbia Univ, New York, NY; 2) Columbia Genome Ctr, Columbia Univ, New York, NY; 3) New York State Psychiatric Institute, New York, NY; 4) Mt. Sinai School of Medicine, New York, NY.

Working memory (WM) deficit is a primary cognitive impairment in schizophrenia. In recent studies (Weinberger et al. 2001, Egan et al. 2001), a functional polymorphism in catechol-O-methyl transferase (COMT) gene was suggested as a strong genetic candidate for individual variance in WM ability, as COMT genotype discriminates performance on the Wisconsin Card Sorting Test (WCST). Because the WCST involves many cognitive components in addition to WM ability, more specific WM tests are required to evaluate the above hypothesis. Here we report results from an ongoing study to identify genes affecting WM ability. Healthy adults were tested on several standard WM tests, including spatial delayed response test, word serial position test, n-back test, and letter-number sequencing test. Wais-III vocabulary test and questionnaires were used to control for IQ impact and psychopathology. Saliva swabs provide a source of genomic DNA for genetic analyses. Preliminary analyses were conducted with the first 274 samples to examine the relation between COMT genotype and WM ability. With the exception of the letter-number sequencing test (ANOVA  $p=0.002$ ), no other significant relation was found between performance scores and COMT genotype. The letter-number sequencing test requires more mental manipulation than other WM tests, and consequently may involve greater executive cognitive processing demands. This result suggests that COMT genotype may be related to other components of executive function in letter-number sequencing test and WCST, besides WM ability. To test this hypothesis, additional subjects are being tested on WCST together with the WM tests. Additional candidate genes for WM will be tested as we simultaneously expand sample size. Subjects with extreme scores will be sequenced to identify new genetic candidates for WM function. Genes implicated in WM function will then be evaluated for disease-related genetic variation in a large schizophrenia sample.

**A High Throughput Genetic Association Study of Tractable Genes in Asthma.** *S.G. Pillai<sup>1</sup>, P.L. St. Jean<sup>1</sup>, D.P. Yarnall<sup>1</sup>, M.J. Wagner<sup>1</sup>, S.S. Sundseth<sup>1</sup>, R.M. DeMent<sup>1</sup>, J.H. Riley<sup>1</sup>, S.X. Seghal<sup>1</sup>, T.K. Fleming<sup>1</sup>, S.N. Hill<sup>1</sup>, G.C. Bennett<sup>2</sup>, S.R. Brewster<sup>1</sup>, E.C. Meldrum<sup>3</sup>, I.J. Purvis<sup>1</sup>, L.T. Middleton<sup>1</sup>, D.K. Burns<sup>1</sup>, N.K. Spurr<sup>1</sup>, A.D. Roses<sup>1</sup>, D.A. Hosford<sup>1</sup>, S.L. Chissoe<sup>1</sup>.* 1) Genetics Research, Glaxo SmithKline, RTP, NC, 27709; 2) GSK Corporate Intellectual Property, RTP, NC, 27709; 3) GSK Center for Excellence in Drug Discovery, Stevenage, UK.

Asthma is the most common chronic childhood disease and it affects a substantial adult population. Hence asthma carries significant direct and indirect economic cost. Several studies have reported linkage or association of asthma to various loci and candidate genes using traditional linkage and candidate gene approaches, but none of these discoveries have yet resulted in new therapeutics for asthma. The pharmaceutical industry focuses on a few gene families -tractable genes- which have a track record of supporting development of successful small molecules into therapeutics. Tractable genes relevant to human disease are the keys to the discovery of new effective medicines for diseases with unmet medical needs. With recent advances in the understanding of the human genome sequence and introduction of high throughput genotyping technologies, it is now possible to undertake large-scale association studies using specific genes. We conducted an association study of 5 asthma phenotypes using SNPs in >1200 tractable genes, carefully selected using bioinformatics approaches. Samples from 294 asthma families with at least one asthmatic child, collected from Denmark and Minnesota were genotyped for 2599 single nucleotide polymorphisms (SNPs) from 1200 tractable genes using a high throughput genotyping platform. A total of 3.5 million genotypes were generated and the data were analyzed using transmission disequilibrium test. Preliminary analysis demonstrated 350, 75, 40 and 8 SNPs with a p value of 0.05, 0.01, 0.005 and 0.001 from 259, 59, 33 and 8 genes with significant association with any of the 5 asthma phenotypes respectively. We are following up positive associations using an independent collection of ~500 families from the Genetics of Asthma International Network (GAIN).

**Transcobalamin II polymorphisms influence plasma levels of vitamin B12 but are not major risk factors for neural tube defects.** *F. Pangilinan<sup>1</sup>, D. Swanson<sup>1</sup>, A. Weiler<sup>1</sup>, V. O'Leary<sup>2</sup>, A. Parle-McDermott<sup>2</sup>, M. Conley<sup>3</sup>, C. Cox<sup>3</sup>, T. Frey<sup>1</sup>, A. Molloy<sup>2</sup>, P. Kirke<sup>4</sup>, J. Mills<sup>3</sup>, J. Scott<sup>2</sup>, L. Brody<sup>1</sup>.* 1) National Human Genome Research Institute; 2) Dept. of Biochemistry, Trinity College-Dublin; 3) National Institute of Child Health and Human Development; 4) Health Research Board, Dublin.

Genetic and environmental factors contribute to the etiology of neural tube defects (NTDs). Lower than average maternal plasma levels of cobalamin (vitamin B12) have been associated with NTDs. Bio-available vitamin B12 is carried in plasma by transcobalamin II (TCII). Amniotic fluid levels of TCII are altered in pregnancies affected by NTDs and in unaffected pregnancies in women who have previously given birth to a child with an NTD. Afman and colleagues reported reduced vitamin B12 binding by TCII as a risk factor for NTDs, and Miller and colleagues reported altered levels of holo-TCII due to the TCII P259R polymorphism.

To evaluate the role of TCII polymorphisms in NTD risk, we genotyped two promoter SNPs and four nonsynonymous coding SNPs in 407 NTD cases, their parents and population matched controls. No significant associations between NTD risk and SNP genotypes were observed. To test whether TCII genotype influences plasma vitamin B12 levels we genotyped 236 normal pregnant women for each of the six TCII SNPs. Mean plasma B12 levels were correlated with TCII P259R genotype (TCII 259PP 289.1 87.7 pg/ml, TCII 259PR 315.1 109.8 pg/ml, TCII 259RR 371.6 142.2 pg/ml, ANOVA  $p = 0.0006$ ). Plasma B12 levels were consistent with an allele dosage effect (test for linear trend  $p = 0.0001$ ). An independent, pooled group ( $n = 868$ ) was similarly analyzed and again a correlation between mean plasma B12 levels and TCII P259R genotype was observed ( $p = 0.037$ ). We conclude that although the examined TCII variants do not appear to contribute to NTD risk, the TCII P259R polymorphism may have a significant impact on other aspects of human health through its affect on plasma vitamin B12 levels.

**SNP, cSNP and Indel Associations with Kawasaki Disease(KD) Susceptibility and Outcomes.** *R.M. Cantor-Chiu<sup>1</sup>, J. Yuan<sup>1</sup>, C. Shimizu<sup>2</sup>, S. Cheng<sup>3</sup>, L. Steiner<sup>3</sup>, H. Shike<sup>2</sup>, S.L. Napier<sup>1</sup>, J.W. Newburger<sup>4</sup>, J.C. Burns<sup>2</sup>.* 1) Human Genetics, UCLA Schl of Med, LA, CA; 2) Pediatrics, UCSD Schl of Med, La Jolla, CA; 3) Roche Mol Sys, Alameda, CA; 4) Pediatrics, Boston Childrens Hosp, MA.

KD is an acute childhood vasculitis that causes potentially life-threatening coronary artery aneurysms (CAA) in 25% of untreated patients. It is likely triggered by infectious agents, but a 5-fold increased incidence in Asians (A) vs. Caucasians (C) (.001 vs. .0002), and a sibling relative risk of 10 in C, strongly suggest the effects of genes. However, the low KD incidence coupled with a low sibling recurrence risk (.002) makes the multiplex families, necessary for a powerful linkage study, rare. Thus, a family-based KD association study is in progress. 105 SNPs, cSNPs and indels in 66 genes, previously implicated in inflammation and/or cardiovascular disease, were genotyped with multi-locus assays (Roche Mol Sys) in a 15% A and 85% C sample of trios (a KD child and both parents). Analyses of the current 130 trios found preliminary evidence of : 1)12 polymorphisms more frequent in A than C ( $p < .007$ ), indicating possible KD genes, 2) no detectable HW disequilibrium of polymorphisms in C, 3)preferential transmission of chemokine receptor 5 (CCR5) ( $p = .02$ ), glycoprotein IIIa ( $p = .02$ ), complement component 5 ( $p = .05$ ), apolipoprotein(a) (LPA) ( $p = .05$ ) and hepatic lipase ( $p = .05$ ) polymorphisms to KD patients by exact transmission disequilibrium tests (TDT), 4) preferential transmission of CCR5 ( $p < .005$ ), LPA ( $p < .007$ ), tumor necrosis factor( $p < .03$ ), and interleukin 6 (IL6) ( $p < .05$ ) haplotypes to KD patients by exact multi-allelic TDT analyses, 5)preferential transmission of polymorphisms in the beta-2-adrenergic receptor ( $p < .02$ ), and IL6 ( $p < .03$ ) to 58 KD patients with CAA. These preliminary findings are not corrected for multiple testing. However, they provide specific hypotheses for analysis in this expanding sample of KD trios. More generally, they demonstrate the value of this family-based association design for identifying genetic contributions to infectious disorders having a low incidence coupled with a low sibling recurrence risk.

Program Nr: 2090 from 2003 ASHG Annual Meeting

**Comparative Analysis of Genome-wide Association Studies Targeting Various Complex Human Diseases and Phenotypic Traits.** *A. Braun, M. Longdown, R. Roth, S. Mah, C.R. Hoyal, G. Marnellos, R. Reneland, M.R. Nelson, S. Kammerer.* Sequenom, Inc., San Diego, CA.

Whole genome association studies using single nucleotide polymorphisms (SNPs), the most abundant variations of the genome, were proposed to identify genes that underlie complex traits. We have developed assays and tested over 220,000 public domain SNPs, with a focus on gene-based regions. Of these, over 130,000 SNPs were confirmed as detectably polymorphic (minor allele frequency > 2%) in at least one population. Approximately 105,000 of these are predicted to have unique map positions in the genome. As a large proof-of-concept study, we employed up to 80,000 of these validated SNP reagent sets in a high-throughput approach using case and control DNA pools. More than 800 associations were identified for 12 common diseases or disease-related traits (breast cancer, prostate cancer, lung cancer, melanoma, diabetes type 2, osteoarthritis, schizophrenia, hypertension, HDL-c, bone mineral density, central fat, and general morbidity). We employ a follow-up strategy that is crucial for distinguishing real and reproducible genetic effects from non-reproducible minor effects and false positives. This includes replication of associations in independent samples, analysis of densely spaced SNPs in the regions of interest, re-sequencing to identify potential causal variants, the development of gene-to-disease hypotheses, which lead to functional experiments. Among the discoveries were several genes previously known to be involved in these traits (e.g. PPAR, CETP, AGC1, CD44). Replicable results and the demonstration of functional changes in these new disease targets represent entry points for the development of novel prognostics, clinical diagnostics and potentially treatment interventions.

**Regulatory genes of NFB and their effect on IBD.** *N.P.S. Crawford, M.R. Eichenberger, T.M. McAuliffe, S. Galandiuk.* Digestive Surgery Research Lab, University of Louisville, Louisville, KY.

Inflammatory bowel disease (IBD) encompasses several chronic inflammatory disorders, and is commonly subdivided into ulcerative colitis (UC), Crohns disease (CD), and indeterminate colitis (IC). Nuclear factor (kappa)B (NFB), a transcription factor that is abnormally active in IBD, up-regulates production of pro-inflammatory molecules in response to a variety of stimuli. The Toll-like receptor (TLR) pathway is a prominent NFB activation pathway and is involved in the innate immune response to many pathogens. We hypothesize that mutations in the genes of critical regulatory proteins within these pathways will play a key role in the pathogenesis of IBD. Mutations of these genes will cause abnormal protein function or expression, which in turn will lead to excessive NFB activity and the chronic inflammation characteristic of IBD. We have initially focused on identifying polymorphisms in the regulatory and amino acid coding regions of the *IB* and *IRAK-M* genes, which are near or within IBD susceptibility loci 4 and 2 respectively.

To date, we have found and characterized polymorphisms in both the *IB* and the *IRAK-M* genes. 387 individuals were genotyped (80 controls, 146 CD, 115 UC and 46 IC) for a C/T polymorphism located 29bp upstream of *IB* exon 4. All three sub-groups of IBD had a higher frequency of the C allele (82%CD, 79% UC and 88%IC) as compared to controls (75%). The comparison between controls and the IC group was significant ( $\chi^2 = 5.4$ ,  $p = 0.02$ ). Within *IRAK-M* we identified the G14339A SNP which is responsible for a valine to isoleucine amino acid substitution. No significant differences between the allele frequencies were found.

These findings suggest that *IB* is involved in the pathogenesis of IBD. We will further analyze other genetic polymorphisms of not only *IB* but *IB* and *IB* as well. Further characterization of polymorphisms within *IRAK-M* will be needed to determine if it is involved in IBD susceptibility.

**Identification of molecular variants at the promoter region of the human alpha7 neuronal nicotinic acetylcholine receptor subunit gene but lack of association with schizophrenia.** *C.-H. Chen<sup>1, 2</sup>, H.-M. Liao<sup>1, 2</sup>, C.-H. Li<sup>1, 2</sup>*. 1) Dept Psychiatry, Tzu-Chi General Hosp, Hualien City, Taiwan; 2) Institute of Human Genetics, Tzu-Chi University, Hualien City, Taiwan.

The human alpha7 neuronal nicotinic receptor subunit gene is considered as a candidate gene for P50 sensory gating deficit in schizophrenic patients. Because P50 sensory gating deficit is a common neurophysiological dysfunction in subjects with schizophrenia spectrum disorders, it is conceivable to hypothesize that the human alpha7 neuronal nicotinic receptor subunit gene might be a susceptible gene for schizophrenia. We searched for mutations at the promoter region of the human alpha7 neuronal nicotinic receptor subunit gene and performed genetic association study in 249 unrelated Han Chinese schizophrenic patients and 273 nonpsychotic subjects from Taiwan. Two molecular variants were identified, designated g.213G>A and g.324A>G, respectively. The g.-213G>A variant was found to obliterate a putative NF-1 transcription factor binding site using computer analysis. One out of 249 patients was detected to be a heterozygote of this variant, but none from 273 controls. The g.-324A>G variant was also very rare in both patients and controls, only one heterozygote of this variant was identified in 249 patients and 273 controls, respectively. Our data do not support the human alpha7 neuronal nicotinic receptor subunit gene as a susceptible gene for schizophrenia in our population.

**Association of ARVCF gene on chromosome 22q11 with schizophrenia.** *H.-Y. Chen*<sup>1</sup>, *J.-I. Yeh*<sup>2</sup>, *M.-Y. Li*<sup>1</sup>, *C.-H. Chen*<sup>1, 3</sup>. 1) Institute of Human Genetics, Tzu-Chi University, Hualien City, Taiwan; 2) Department of Family Medicine, Tzu-Chi University, Hualien City, Taiwan; 3) Department of Psychiatry, Tzu-Chi University, Hualien City, Taiwan.

Patients with chromosome 22q11 microdeletion have increased liability to schizophrenia, suggesting that 22q11 locus may harbor schizophrenia susceptible gene(s). ARVCF is a new catenin family gene mapped to 22q11, which has potential function of mediating cell-to-cell communication and intracellular signal transduction. To test if ARVCF gene is a schizophrenic susceptible gene, we systematically screened the ARVCF gene for mutations in Han Chinese schizophrenic patients from Taiwan. Ten single nucleotide polymorphisms (SNPs) were identified, including four nonsynonymous SNPs, V175A, R196Q, P220L, and R915Q. Genetic association study of these 4 missense SNPs with schizophrenia showed excessive homozygosity of P220 in 102 patients compared to 103 controls (odds ratio = 2.38, 95% confidence interval = 1.30-4.35,  $\chi^2 = 8.01$ ,  $p = 0.0046$ ). The association was replicated by increasing the sample size to 319 patients and 316 controls (odds ratio = 1.5, 95% confidence interval = 1.08-2.07,  $\chi^2 = 6.02$ ,  $p = 0.014$ ). Our data suggest that P220 allele of ARVCF is modestly associated with schizophrenia, which may play a modifying role in the pathogenesis of schizophrenia.

**Positional cloning of a novel gene associated with persistent Hepatitis B Virus Infection.** *A.J. Frodsham<sup>1</sup>, S. Best<sup>1</sup>, S. Lobello<sup>2</sup>, C. Venturi-Pasini<sup>2</sup>, H. Thomas<sup>3</sup>, M. Chiaramonte<sup>2</sup>, M.R. Thursz<sup>3</sup>, A.V.S. Hill<sup>1</sup>.* 1) The Wellcome Trust Centre for Human Genetics, University of Oxford, U.K; 2) Istituto di Medicina Interna, Università di Padova, Italy; 3) Imperial College School of Medicine, St Mary's Hospital, London, UK.

Persistent HBV carriage affects over 350 million people in the world and the end stage sequelae of infection account for 1 million deaths each year. The major global health burden is located where rates of HBV persistence are at their highest in regions such as South East Asia and sub-Saharan Africa. Evidence of the involvement of host genetics has been provided by twin studies and association studies with both HLA and non-HLA genes.

A whole genome scan using families from Italy was completed. A locus on chromosome 6q27 was identified that shows strong evidence of linkage to outcome of infection. Fine mapping and further analysis identified several polymorphic markers within a region of less than 150kb that show evidence of association with persistent infection ( $P < 0.05$ ). Examination of the haplotypes in this region also showed a highly significant association with outcome of infection ( $P < 0.001$ ). Analysis of the extent of LD in this region suggests that significant LD extends over at least 250kb.

A strategy of sequencing and SNP genotyping was used to investigate and exclude the closest known gene to this region. LD analysis also confirmed that SNPs in this gene are in different haplotype blocks than the associated markers. Examination of the genomic sequence in the region containing the markers revealed no previously identified genes. However several ESTs (expressed sequence tags) map into this region. Via cDNA amplification and sequencing of liver tissue samples, we have identified a large open reading frame in this region corresponding to an 895 amino acid protein and further analysis is underway to elucidate the role this protein plays in determining the outcome of HBV infection.

**Familial and sporadic endometriosis are genotypically different: The role of liver detoxification genes in the pathophysiology of endometriosis.** *I. Flores<sup>1</sup>, E. Rivera<sup>1</sup>, S. Abac<sup>1</sup>, S. Abreu<sup>2</sup>, C. Rios-Bedoya<sup>2</sup>, A. Santiago-Cornier<sup>3</sup>.*  
1) Department of Microbiology, Ponce School of Medicine, Ponce, PR; 2) Epidemiology and Biostatistics Core Program, Ponce School of Medicine, Ponce, PR; 3) Department of Biochemistry, Ponce School of Medicine, Ponce, PR.

Endometriosis is a multifactorial disease characterized by dysmenorrhea, dyspareunia, and infertility. The cause of this common gynecologic condition remains elusive, although increasing evidence supports the role of genetic susceptibility in disease etiology. Endometriosis has been shown to be associated with polymorphisms in liver detoxification enzymes, including glutathione S transferase (GST), N-acetyl transferase 2 (NAT-2) and cytochrome P450 (CYP). This study was conducted to determine whether associations in these three genes are also found in a Puerto Rican population of endometriosis patients. Cases of endometriosis (n=112) and controls (n=59; females who had no endometriosis as shown by surgery and male volunteers) were genotyped according to standard protocols using either PCR amplification alone or PCR-RFLP. No statistically significant differences were observed between cases and controls in any of the genes studied. However, when endometriosis patients were grouped into familial and sporadic patients, patients with a family history were significantly more likely to have the GST-T1 null mutation than either sporadic patients (p=.002) and controls (p=.035). Moreover, only familial endometriosis was significantly associated with the slow acetylator phenotype of NAT-2 (p=.018; OR=6.6). This study provides evidence for the differentiation of familial and sporadic endometriosis as two different diseases at the genetic level, and supports a role of environmental toxicant exposure in the development of this disease.

**Association of the serotonin receptor 5-HT<sub>5A</sub> with autism.** A.M. Coutinho<sup>1</sup>, G. Oliveira<sup>2</sup>, C. Fesel<sup>1</sup>, C. Marques<sup>2</sup>, A. Ataíde<sup>2</sup>, T. Miguel<sup>2</sup>, L. Borges<sup>2</sup>, A.M. Vicente<sup>1</sup>. 1) Instituto Gulbenkian Ciencia, Oeiras, Portugal; 2) Hospital Pediatrico de Coimbra, Coimbra, Portugal.

Autism is a neurodevelopmental disorder with a complex genetic etiology. Consistent linkage has been found to chromosome 7q, at 7q31 and at 7q35-36 where loci involved in language impairment and restrictive-repetitive behaviors are located. The serotonin receptor 5A (5-HT<sub>5A</sub>), which is expressed only in the central nervous system and thus likely mediates the central effects of serotonin, is located on 7q36.1. Association of the *HTR5A* gene with affective disorders has been reported, and knockout mice for this gene show behavior alterations. We tested the involvement of *HTR5A* in autism susceptibility and in the specific autism-associated phenotype indexed by the total scores for algorithm items of the area of Restricted, Repetitive Behavior and Interests of the Autism Diagnostic Interview-Revised (ADI-R) (D total score). Three SNPs were tested (-19G/C and -18C/T in 5' UTR, and 12A/T in exon 1) in 194 nuclear families (including 202 probands). Using the Transmission Disequilibrium Test an association with the marker -18C/T was found ( $P=0.012$ ), with allele -18C more often transmitted than -18T ( $\chi^2=6.1$ , 1 df,  $P=0.0136$ ), and a preferential paternal transmission ( $\chi^2=7.2$ , 1 df,  $P=0.0073$ ) suggestive of maternal imprinting. Haplotypes of the three markers were also associated with autism ( $P=0.033$ ). Kruskal-Wallis one-way ANOVA was used to test the association of *HTR5A* genotypes with the Restricted, Repetitive Behavior and Interests scores (D total score). The exon 1 marker 12A/T was found to be associated with the D total score distribution, with higher mean scores, which reflect an increased severity of the abnormal behavior, associated with genotype 12A/12A ( $H=6.26$ , 1 df,  $P=0.0124$ ) and lower mean scores associated with genotype 12A/12T ( $H=11.3$ , 1 df,  $P=0.0008$ ). Our data suggest a role of *HTR5A* variants in the susceptibility to autism and in the determination of repetitive and stereotyped behaviors, likely including complex interactions with other molecules of the 5-HT system.

**Human variants of SCN1, a modifier gene on chromosome 1q21.** *D.A. Buchner, M. Trudeau, M.H. Meisler.*  
Human Genetics, University of Michigan, Ann Arbor, MI.

The severity of many Mendelian disorders is influenced by genetic background. We recently identified a modifier gene in the mouse, *Scnm1*, that converts a chronic movement disorder into a lethal disease. The primary mutation (*med<sup>J</sup>*) changes a splice donor site of the mouse sodium channel gene *Scn8a* (*Na<sub>v</sub>1.6*) and results in a mixture of wildtype transcripts (10%) and exon-skipped mutant transcripts (90%). The modifier mutation was identified using sequence databases to compare resistant and susceptible inbred strains. A premature stop codon was identified in the nuclear zinc finger protein, *SCNM1*. The effect of the modifier mutation is to reduce the proportion of correctly spliced sodium channel transcript to 5% of total, which is below the threshold for survival. The identity of the modifier gene was confirmed by transgenic rescue of the *C57BL/6J-med<sup>J</sup>/med<sup>J</sup>* lethal phenotype. A survey of 36 inbred strains demonstrated that the R187X allele was present only in the closely related C57 and C58 related strains, indicating that the mutation arose in a common ancestor approximately 80 years ago. The *SCNM1* protein contains a bipartite nuclear localization signal, a U1C-like zinc finger domain, and a C-terminal acidic domain, and is predicted to function in 5' splice site selection.

The naturally occurring variant of *Scnm1* in strain *C57BL/6J* was phenotypically unrecognized until it was combined with the second-site mutation in *Scn8a*. The human ortholog, *SCNM1*, is located on chromosome 1q21. To determine whether variants of *SCNM1* are present in the human population, we have screened 179 genomic DNA samples by exon amplification and conformation sensitive gel electrophoresis. Nine individuals (4%) were heterozygous for four different coding variants. Two variants are nonconservative substitutions in evolutionarily conserved residues. By analogy with the *C57BL/6J* mouse, we suggest that functionally compromised variants of *Scnm1* may contribute to the severity of human disorders caused by splice site mutations in unlinked genes.

**Association of *IL4R* haplotypes with Multiple Sclerosis.** *D.B. Mirel*<sup>1</sup>, *J. Wang*<sup>1</sup>, *H.A. Erlich*<sup>1</sup>, *S.L. Hauser*<sup>2</sup>, *L.F. Barcellos*<sup>2</sup>, *J.R. Oksenberg*<sup>2</sup>. 1) Dept. of Human Genetics, Roche Molecular Systems, Inc, Alameda, CA; 2) Dept. of Neurology, University of California, San Francisco; San Francisco CA.

We have investigated, in 332 single-case families, the association of multiple sclerosis (MS) with ten SNPs in the *IL4R* gene. *IL4R* encodes a subunit of the interleukin-4 receptor, a molecule important to cytokine signaling and T-helper cell development. We have genotyped all individuals at eight SNPs within the *IL4R* coding region: I50V, N142N, E375A, L389L, C406R, S478P, Q551R, and S761P, and at two promoter SNPs: C(-3223)T and T(-1914)C. We restricted our study to families where the subject was affected with either the Relapsing Remitting (RR) or Secondary Progressive (SP) course of MS, and stratified families according to presence of the MS-predisposing *HLA*-DR2 haplotype (DRB1\*1501-DQB1\*0602).

The association of individual *IL4R* SNPs with MS was examined using the Transmission-Disequilibrium Test. None of the ten SNPs individually showed an association, even after *HLA*-DR2 stratification. However, as there is strong linkage disequilibrium among these SNPs we could infer the transmission of ten-locus haplotypes in families using the TRANSMIT program of Clayton (1999). By identifying haplotypes (complex alleles) in the MS families, stratified for *HLA* genotype, we have observed significant evidence of association of the *IL4R* gene to MS. In particular, we have identified a specific susceptibility haplotype, and observe that this complex allele confers MS risk in individuals not carrying the *HLA* DR2 genotype (odds ratio = 1.4, adjusted P = 0.026). The MS susceptibility *IL4R* haplotype identified here is distinct from the type-1 diabetes protective haplotype we have previously described (Mirel et al. 2002; Bugawan et al. 2003). These findings further support an important role for the *IL4R* gene and its alleles in immune-related disease susceptibility, and illustrate the value of utilizing multi-SNP haplotype information in association studies.

**Differential gene expression in central human retina by a comparative microarray approach.** *D. Hornan<sup>1</sup>, S. Peirson<sup>2</sup>, A. Hardcastle<sup>1</sup>, A. Bird<sup>1</sup>, S. Bhattacharya<sup>1</sup>, R. Foster<sup>2</sup>, R. Molday<sup>3</sup>, A. Webster<sup>1</sup>.* 1) Department of Molecular Genetics, Institute of Ophthalmology, London, UK; 2) Department of Integrative and Molecular Neuroscience, Imperial College London, UK; 3) Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada.

The central human retina contains many more cone photoreceptors than the peripheral retina. Cones are essential for precise vision and are known to express specific opsins and other proteins that form part of the phototransduction cascade. However, much more is known about gene expression in the rod-rich peripheral than the cone-rich central retina.

We used human 2mm foveo-macular and 4mm macular punches, and sections of mid-peripheral retina to study differential gene expression. By combining multiple microarray experiments with quantitative PCR and cutting-edge statistical and bioinformatic analyses, we identified both known and unknown retinal genes.

Two novel retinal genes are expressed at levels approaching that of red/green cone opsin in the central human retina. Two other genes, also found to be over-expressed in central human retina, are involved in the cellular response to stress.

The genes uncovered in this study will provide insight into the role of the central retina in precise vision. They are also likely to be involved in diseases of the macula and fovea such as age-related macular degeneration.

Program Nr: 2100 from 2003 ASHG Annual Meeting

**-sarcoglycan, a gene in which variants cause Myoclonus-Dystonia Syndrome, may also contain Schizophrenia vulnerability variants.** *M. Mineta*<sup>1</sup>, *H. Ishiguro*<sup>2</sup>, *M. Egan*<sup>3</sup>, *J. Taubman*<sup>1</sup>, *B. Kolachana*<sup>3</sup>, *T. Rowe*<sup>1</sup>, *R. Lipsky*<sup>1</sup>, *G.R. Uhl*<sup>2</sup>, *D.R. Weinberger*<sup>3</sup>, *D. Goldman*<sup>1</sup>. 1) Laboratory of Neurogenetics, NIAAA / NIH, Rockville, MD; 2) Molecular Neurobiology Branch, NIDA / NIH, Baltimore, MD; 3) Clinical Brain Disorders Branch, NIMH / NIH, Bethesda MD.

The -sarcoglycan (SGCE) gene encodes transmembrane components of the dystrophin-glycoprotein complex. SGCE loss-of-function mutations cause a myoclonus-dystonia syndrome (MDS) that often includes prominent psychiatric abnormalities. Since the SGCE gene is localized to the Chr 7q21-q22 region that has been implicated in schizophrenia in previous genome scans, SGCE variants are candidates to play roles in schizophrenia. Imprinting at this locus is supported by findings that include MDS transmission through predominantly paternally-inherited alleles. We now report that sixteen-locus transmission disequilibrium testing (TDT) in 114 Caucasian schizophrenia trios provides evidence for significant linkage and association ( $p < 0.005$ ) with a ca. 100kb conserved haplotype block that includes SGCE. We are evaluating the effects of SGCE haplotypes on expression to determine whether this gene or surrounding genes in this haplotype block harbor variants that contribute to susceptibility to schizophrenia.

**A complete map of sequence variation in the DTNBP1 high-risk haplotype from schizophrenics in the Irish Study of High Density Schizophrenia Families.** *B. Riley<sup>1</sup>, D. Thiselton<sup>1</sup>, B. Wormley<sup>1</sup>, R. Ribble<sup>1</sup>, G. Frank<sup>1</sup>, E.J.C.G. van den Oord<sup>1</sup>, D. Walsh<sup>2</sup>, F.A. O'Neill<sup>3</sup>, K.S. Kendler<sup>1</sup>.* 1) Virginia Institute for Psychiatric and Behavioral Genetics, Depts. of Psychiatry and Human Genetics, Virginia Commonwealth University, Richmond, VA; 2) The Health Research Board, Dublin, Ireland; 3) Department of Psychiatry, Queens University, Belfast, Northern Ireland.

We have previously shown association of markers in the dystrobrevin binding protein 1 (DTNBP1) gene with schizophrenia, identified a haplotype block of 30.1 Kb covering exons 1 to 5 of this gene, and identified one high risk haplotype from the six common haplotype structures within this block in the Irish Study of High Density Schizophrenia Families (ISHDSF). Another of the six common haplotypes has been identified as high risk in a sample of German families and trios. We have selected all genetically independent, narrowly-defined ISHDSF cases with the Irish high risk haplotype and controls with the most closely related haplotype for sequence analysis to identify variation that maps specifically to the high-risk haplotype and that may account for the increased risk, as well as to catalogue the complete range of sequence variation within this region. At the time of submission, with >90% of the sequence complete, we have identified 37 SNPs, with average SNP density across the region of 1.6/Kb. Although higher than the genome-wide average, this is likely a result of inclusion of the numerous repeat motifs occurring in this gene in our analyses, rather than an indication of higher than normal variation in this gene. We have also identified 6 additional polymorphisms, three ins/del polymorphisms of 4, 6 and 10 bp, a mononucleotide T-repeat, a dinucleotide A/C repeat and a complex 31 bp sequence variant. Of these, two (5.4%) of the SNPs and one allele of the T-repeat map specifically to our high-risk haplotype. All three of these polymorphisms map to a 3 Kb segment of intron 3. Polymorphisms from the map of haplotype-specific variation in the ISHDSF provide candidate are being tested in family, case-control and triads samples from Ireland at the time of submission.

**Gene Expression Changes in Suicides with and without Major Depression.** *G. Turecki*<sup>1</sup>, *A. Sequeira*<sup>1</sup>, *F. Gwadry*<sup>2</sup>, *L. Canetti*<sup>1</sup>, *Y. Gingras*<sup>1</sup>, *J. Ffrench-Mullen*<sup>2</sup>. 1) Dept Psychiatry, Douglas Hosp, McGill Univ, Montreal, PQ, Canada; 2) Gene Logic Inc, Gaithersburg, MD.

Classical binding and biochemical approaches have pointed out to a large number of possible biological markers for suicide. However, these classical approaches have produced conflicting results. Gene expression profiling at the level of mRNA by means of cDNA microarrays offers the possibility to study the expression patterns of thousand of genes in parallel. The purpose of this study was to test the hypothesis that brain gene expression profiles are associated specifically with suicide and with major depression. Total mRNA was isolated from 7 selected brain cortical areas from 20 suicide cases (10 with and 10 without major depression) and 10 matched controls using the Affymetrix HG-U133 chip set. At the  $p < 0.001$  level, the number of fragments differentially expressed was of 685 between controls and suicides with major depression, 106 between controls and suicides and 233 between suicides with major depression and suicides without major depression. Two biologically relevant genes were selected for further investigation based on expression patterns that were significantly and consistently different between groups. These were Spermine/Spermidine N1-acetyltransferase (SAT) gene and the insulin-like growth factor binding protein (IGFBP 7) gene. SAT is the rate-limiting enzyme in the biosynthesis of polyamines, which play a key role in protein synthesis, cell proliferation, and neuronal plasticity and is implicated in the cellular response to stress. Insulin-like growth factor binding proteins are soluble proteins that regulate IGF (insulin growth factor) availability in body fluids and tissues and that modulate IGF binding to its receptors. SAT and IGFBP7 genes alterations were confirmed by means of RT-PCR and immunohistochemistry in the same brain samples. In conclusion, gene alterations investigation in post-mortem human brains by means of microarray analysis have produced consistent results and showed specific differences associated with suicide and major depression.

**Characterization of a Translocation Breakpoint on Chromosome 20 in a Pair of Autistic Twins.** *R. Sultana*<sup>1</sup>, *W.H. Raskind*<sup>2,5</sup>, *C.M. Disteché*<sup>3</sup>, *F. Cortes*<sup>7</sup>, *F. de la Barra*<sup>8</sup>, *G.D. Schellenberg*<sup>2,4,6</sup>, *E.C. Villacres*<sup>1</sup>. 1) Psychiatry & Behavioral Sciences; 2) Medicine; 3) Pathology; 4) and Neurology, Univ Washington; 5) MIRECC; 6) and GRECC at SVAMC Seattle, WA; 7) Genetic and Metabolic Diseases Unit, INTA; 8) and Psychiatry and Mental Health Department, Univ Chile.

A pair of autistic twins with a t(7;20) (q11.2; p11.2) was analyzed to identify the chromosome 20 gene disrupted by the translocation. Previously a novel gene (AUTS2) that spans this 7q11.2 breakpoint in these twins was cloned. To determine the position of chromosome 20 breakpoint we generated somatic cell hybrids between a lymphoblastoid cell line from one of the twins and Chinese Hamster Ovary (CHO) cells. Two hybrids, one with the derivative chromosome 7 and the other with derivative chromosome 20, were used to narrow the region of this breakpoint. STS markers from BAC clones that had been mapped to 20p11.2 by FISH were selected for STS content mapping of the two hybrid cell lines. Initial STS content mapping of the hybrids mapped the 20p11.2 breakpoint to a 27 Mb region of NT\_001387. Additional primer pairs were designed to further narrow the breakpoint to a 61 Kb chromosome 20 region. To amplify a fragment spanning this breakpoint five chromosome 20 primers from the 61 Kb region were used in long-range PCR, each paired with a chromosome 7 primer within 500 bp of the breakpoint. The PCR fragment amplified in this manner was sequenced and the location of the breakpoints determined on chromosomes 7 and 20 sequences by BLAST analysis. The breakpoint maps to position +/- 26008190 and +/- 25685711 of the chromosome 20 sequence in a duplicated segment of chromosome 20. Which one of these two chromosome 20 sites is disrupted by this breakpoint has yet to be determined. Sequencing of a larger DNA fragment across the breakpoint and analysis of single nucleotide polymorphic sites present in the two duplications will give information about the exact location of this breakpoint within this segmental duplication of chromosome 20. The effect of this translocation on chromosome 20 genes in the vicinity of the breakpoint will be analyzed. Analysis of these patients can lead to the identification of gene defects that cause autism.

**A meta-analysis of association and linkage between the dopamine transporter gene (DAT1) and childhood ADHD.** *I.D. Waldman.* Psychology, Emory University, Atlanta, GA.

Recently, a number of studies have examined association and linkage between candidate genes in the dopaminergic neurotransmitter system and childhood attention-deficit hyperactivity disorder (ADHD). Several studies have demonstrated association and linkage between ADHD and a number of these genes, specifically the dopamine receptor D4 and D5 genes (i.e., DRD4 and DRD5) and the dopamine transporter gene (i.e., DAT1), whereas some studies have failed to replicate these relations. In this study, we examine linkage and association between ADHD and a functional polymorphism in the 3' untranslated region of DAT1 via a meta-analysis of effect sizes derived from transmission disequilibrium test results from 18 independent samples. We tested for association and linkage between DAT1 and ADHD (i.e., whether the relative risk [RR] was significantly greater than 1) across studies, as well as whether there was significant heterogeneity in the effect sizes. We also conducted similar analyses for DRD4 and ADHD to compare the magnitude of effect sizes to that for DAT1. Results from meta-analyses of data from 18 samples suggest association and linkage of both DAT1 and DRD4 with ADHD (RR = 1.14,  $p = .073$  and RR = 1.4,  $p = .004$ , respectively). Despite the similar relative risks for these two genes, their level of significance differed markedly. This is due to the fact that there was little heterogeneity in the effect sizes for DRD4, but substantial heterogeneity in the effect sizes for DAT1, suggesting that systematic differences in the samples and / or methods of these studies may be contributing to differences in the relation of DAT1 and ADHD. In an attempt to explain the heterogeneity in the DAT1 effect sizes, we are testing for the potential moderating role of several variables, including the magnitude of DRD4 effect size, the location of the study (i.e., US / Canada versus UK / Europe / Asia), the proportion of ADHD subtype diagnoses (i.e., Combined versus Inattentive type), the rates of overlapping diagnoses (e.g., of Oppositional Defiant and Conduct Disorder), the proportion of male participants, and the assessment method used (i.e., interview versus questionnaire).

**Identification of autism susceptibility genes on 7q31 using cytogenetic abnormalities.** *J.B. Vincent<sup>1,2</sup>, S. Choufani<sup>2</sup>, D. Kwasnicka<sup>2</sup>, J. Skaug<sup>2</sup>, S.W. Scherer<sup>2</sup>.* 1) Neurogenetics Sect, R30, Ctr Add/Mental Hlth, Toronto, ON, Canada; 2) Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto.

We have attempted to identify candidate autism susceptibility genes by combining evidence from genetic linkage studies of autism, and cytogenetic abnormalities in patients with autism to search for susceptibility genes for autism on chromosome 7. Evidence for a gene or genes for autistic disorder on chromosome 7q has been documented by a number of genome-wide linkage studies. Supporting evidence for an autism susceptibility locus on 7q has come from cytogenetic studies of autism patients with chromosomal abnormalities on 7q. We have now identified four autism patients with chromosomal aberrations mapping to 7q, within the region implicated by the linkage studies: HSC1: t(7;13)(q31.2;q21); HSC2: t(5;7)(q14;q31); HSC3: t(6;7)(p12;q22); HSC4: t(7;11)(q31;q25). Using fluorescence in situ hybridization we have mapped the breakpoints for the patients abnormal chromosomes, and have identified and cloned several novel genes (RAY1/ST7 and TCAG\_4133353, HSC1 and HSC2 respectively) and identified several other known genes (KCND2, NPTX2, FOXP2, for HSC2, HSC3 and HSC4 respectively) that are either disrupted by or are adjacent to the breakpoints. The breakpoints for HSC1, HSC2 and HSC4 are all within 5Mb of each other on 7q31. Using denaturing high performance liquid chromatography and DNA sequence analysis, we have screened a number of the candidate genes for mutations among over 90 multiplex autism families, and have identified a number of rare DNA variants. For KCND2, a gene encoding a brain-specific potassium voltage-gated channel, several amino acid sequence variants were identified in exon 5, including N490D variant present in both affected and one unaffected sibling in one family, and N544S, present in both affected siblings of a second family. We have identified a number of variants within exons of the putative non-coding gene, TCAG\_4133353, which spans the HSC2 breakpoint, including a polymorphic 300bp insertion/deletion. Using RT-PCR we are currently studying expression of genes near the breakpoints to determine whether transcription is being disrupted via a position effect.

Program Nr: 2106 from 2003 ASHG Annual Meeting

**Large-Scale SNP Typing Of Candidate Genes For Susceptibility To Malaria.** *A.J. Walley, C. Aucan, A.V.S. Hill.*  
Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford, United Kingdom OX3 7BN.

Malaria is a global health problem affecting approximately 40% of the world's population, with a prevalence of 300-500 million clinical cases per year. Around 90% of these cases are in sub-Saharan Africa and result in over 1 million deaths per year, predominantly of children. Host genetic factors have been shown to play a central role in resistance mechanisms against malaria infection and clinical malaria, e.g. sickle-cell trait. In order to identify more genes associated with malaria susceptibility, a high-throughput SNP genotyping technique was adopted. Forty-one genes were identified as possible candidates, e.g. genes from the toll-like receptor signalling pathway, suppressors of cytokine signalling and interferon-regulatory factors. Single nucleotide polymorphism (SNP) information was obtained from online databases. However, most SNPs did not have information on allele frequencies in Africans. A total of 202 possible SNPs were used in a two-stage screening strategy to genotype a Gambian case-control study group using the Sequenom MassARRAY assay. The SNP assay PCRs were typically 7-plex, giving a total of 30 sets. Each set was used in an initial screen of 188 severe cases and 188 mild controls and if there was a significant association with a malaria phenotype ( $p < 0.05$ ) this was followed by screening of the remaining 1056 samples, giving a total of 538 severe malaria cases, 338 mild malaria cases and 562 controls. From the first 140 SNP assays, 35% of assays were successful, 23% significantly deviated from Hardy-Weinberg equilibrium, 29% of the SNPs were non-polymorphic in this dataset and 12% of assays failed outright. From this initial data, analysis of 48 SNPs using a 3x2 Pearson's chi-squared test, an association between one of the SNPs and malaria disease status was significant for both genotype ( $\chi^2=14.15$ ,  $p=0.0008$ ) and allele ( $\chi^2=5.74$ , Odds Ratio=1.49 (95% C.I. 1.06-2.09,  $p=0.017$ )). The genotype association remained significant after Bonferroni correction for multiple testing ( $p=0.038$ ).

**Expression profiling in muscle from untreated children with juvenile dermatomyositis (JDM) with pathological calcifications.** *Y.-W. Chen*<sup>1</sup>, *L.M. Pachman*<sup>2</sup>. 1) Center for Genetic Medicine Research, Childrens National Medical Center and George Washington University, Washington, DC; 2) Dept of Pediatrics, The Childrens Mem. Institute for Education and Research, Northwestern University, Chicago, IL.

JDM is the most common pediatric inflammatory myopathy. Children with JDM may develop pathological calcifications (PCa<sup>++</sup>), which contribute to both the morbidity and mortality of this often chronic disease. We established that PCa<sup>++</sup> are associated with: 1) prolonged duration of untreated disease, 2) TNF-308A polymorphism, 3) disease activity. The goal of the study is to identify differentially expressed genes in muscle from JDM patients with and without PCa<sup>++</sup> to understand the molecular mechanisms involved in the process. Muscle biopsies from 10 untreated JDM (5 with PCa<sup>++</sup>, 5 without PCa<sup>++</sup>) and four control children were expression profiled using Affymetrix human U133 chips containing ~42,000 full-length genes and ESTs. Comparisons between JDM +/- PCa<sup>++</sup> and gene changes with p-values less than 0.001 were selected for further analyses. Expression levels from the control samples (changes from baseline) were determined. The results showed that JDM with PCa<sup>++</sup> patients had upregulation of ICAM2, MARKS (myristoylated alanine-rich protein kinase C substrate), ICAM3, and MASP1 (mannan-binding serine protease-1) compared to untreated children with JDM without PCa<sup>++</sup>. Twenty-six genes down-regulated the most prominent (greater than two fold) in the JDM with PCa<sup>++</sup> group were IL-12 receptor B2, and 8 other genes. Comparison with control samples showed that ICAM2 and MARKS were upregulated in JDM with PCa<sup>++</sup>, while ICAM3 and MASP1 were down regulated in JDM without PCa<sup>++</sup>. We concluded that upregulation of adhesion molecules, ICAM-1,-2, -3 may play a role in localizing the site of PCa<sup>++</sup>. Persisting inflammation is associated with PCa<sup>++</sup> in JDM muscle. Contributing factors are: duration of untreated disease; increased TNF- associated with the TNF-308A allele, and decreased expression of the IL-12 receptor. We expect that further dissection of differential expression profiles in JDM may lead to a better understanding of the pathophysiology of PCa<sup>++</sup>.

**Molecular basis of complete C4A and C4B deficiencies in human SLE and kidney diseases.** *Y. Yang<sup>1</sup>, K. Lhotta<sup>2</sup>, E.K. Chung<sup>1</sup>, C.Y. Yu<sup>1</sup>.* 1) Molecular and Human Genetics, Columbus Childrens Research Institute, and Department of MVIMG, OSU; 2) Department of Clinical Nephrology, Innsbruck, Austria.

Complete deficiencies of human complement C4A and C4B are strongly associated with infectious disease, systemic lupus erythematosus (SLE) or kidney diseases. So far, 29 individuals with complete C4A and C4B deficiencies have been identified. The molecular basis of complete C4 deficiency has been determined only in five subjects with three different deleterious mutations, including a 2-bp insertion in exon 29, a 1-bp deletion in exon 20, and a 1-bp deletion in exon 13. In these complete C4 deficient patients, two common HLA haplotypes that affect multiple individuals in different families have been revealed. To investigate the molecular basis of complete C4 deficiency in these two HLA haplotypes, four families with 7 patients from the Alpine region were recruited. They are either homozygous in HLA A24 Cw7 B38 DR13 or HLA A30 B18 DR7. They have SLE, recurrent hematuria with membranous nephropathy, or Henoch-Schoenlein purpura. Definitive genomic Southern blot analysis and pulsed field gel electrophoresis experiments revealed a single, long mutant C4A gene in three patients with the HLA B38-DR13 haplotype, and an unusual bimodular RCCX module with two short mutant C4B genes and with two steroid CYP21B genes in four patients with the HLA B18-DR7 haplotype. Long-range PCR strategies were applied to amplify and clone the mutant C4A and C4B genes. The three mutant C4 genes were sequenced to completion except the endogenous retrovirus in the long C4A gene. A novel 2-bp deletion in exon 13 was discovered in the mutant C4A genes. The identical G to A nucleotide substitution at the splice junction of intron 28 was found in the two C4B mutant genes. Hence, there are two hot-spots with deleterious mutations in C4A<sub>Q0</sub> and C4B<sub>Q0</sub>: one is located at exon 13; the other is located within a 2.6 kb genomic region spanning exon 20 to exon 29. Definitive SSP-PCR and RFLPs have been created to facilitate screening of the C4 gene mutations. This will help determine the prevalence of C4 mutants in infectious and autoimmune diseases more accurately.

**Systemic lupus erythematosus (SLE) is associated with private mutations and public haplotypes of DNASE1 and DNASE1L1.** *D. Xu<sup>1</sup>, M.K. Lee<sup>1</sup>, K. Dalakishvili<sup>1</sup>, Y. Wang<sup>1</sup>, N. Shen<sup>2</sup>, S.L. Chen<sup>2</sup>, J.E. Salmon<sup>3</sup>, M.C. King<sup>1</sup>.* 1) Medical Genetics and Genome Sciences, U Washington, Seattle WA; 2) Institute of Rheumatology, Shanghai, China; 3) Hospital for Special Surgery, New York, NY.

DNASE1 and its homologs, all involved in clearance of DNA from autoantigenic nucleoprotein complexes, are candidate genes for inherited predisposition to SLE. DNASE1 homologs are 40% to 56% identical in protein sequence and differ in chromosome and in cellular localization: for example, DNASE1 (16p13.3) is secreted and DNASE1L1 (Xq28) membrane-bound. DNASE1-deficient mice develop features similar to human SLE and an inherited nonsense mutation in DNASE1 has been reported in two Japanese patients. We evaluated DNASE1 and DNASE1L1 genomic sequences, htSNP-defined haplotype blocks, and message expression levels in Caucasian SLE cases and matched controls and in Chinese SLE patients and their parents. In a Caucasian SLE patient, we identified a novel DNASE1 protein truncating mutation due to a tandem 56-bp duplication and a stop at codon 86. This mutation is not found in other SLE patients or in >1000 controls. DNASE protein activity of this patient, measured by the SRED method, is lower than that of controls ( $P < 0.0001$ ). Furthermore, an unrelated haplotype of DNASE1 ("CGG") is 4-fold more common among Caucasian SLE patients than controls (OR = 4.02; 95% CI [1.51,11.3]), and in Chinese families, a parent with DNASE1\*CGG is more likely to transmit this haplotype to a child with SLE ( $P = 0.03$  by TDT). DNASE1L1 haplotypes defined by htSNPs and a 3'UTR 196-bp deletion differ in frequencies between Caucasian SLE cases and controls ( $P = 0.007$ ). Transcript expression of DNASE1L1 differs very significantly by haplotype (Hotelling  $P < 0.00001$ ) and within each haplotype cluster is lower for SLE cases than controls ( $P = 0.01-0.03$ ). Similar analyses of DNASE1L2 and 1L3 are underway. Inherited variation in the DNASE1 family may play an important role in the pathogenesis of SLE. Integrating patients genotypes at these loci into future clinical trials of recombinant human DNASE1 therapy may be beneficial.

***ASCT2*, key molecule in placental morphogenesis, escapes from imprinting in placenta.** *N. Yahagi*<sup>1</sup>, *H. Asada*<sup>2</sup>, *R. Kosaki*<sup>3</sup>, *T. Takahashi*<sup>1</sup>, *K. Kosaki*<sup>1</sup>. 1) Department of Pediatrics, Keio Univ, Tokyo, Japan; 2) Department of Obst, Keio Univ, Tokyo, Japan; 3) Division of Medical Genetics, Saitama Children's Medical Center Iwatsuki, Japan.

From an evolutionary standpoint, genome imprinting is observed only in mammals (eutherians). Because only the mammals have placenta and that most imprinted genes are expressed in placenta, it is speculated that genomic imprinting co-evolved with the placental system. Furthermore, the conflicting theory states that imprinting regulates the balance between the maternal and fetal nutrients. In terms of nutrient transfer in human placenta, the syncytiotrophoblast cell layer represents the maternal-fetal barrier. Syncytiotrophoblasts are formed by the fusion of cytotrophoblasts with a help of Syncytin, an endogenous retroviral envelope glycoprotein and its receptor *ASCT2*, sodium-dependent neutral amino acid transporter type2. We attempted to determine whether human *ASCT2* is imprinted in placenta in view of its crucial role in the formation of placental barrier and its map location to the vicinity of the known imprinted gene cluster on 19q13. Allelic expression of the *ASCT2* transcript was determined using RT-PCR analysis with a primer pair designed to amplify across the *g/c* single-nucleotide polymorphism within exon 1. In all of the 6 term placental tissue samples shown to be heterozygous for the *g/c* polymorphism, the *ASCT2* gene was expressed by both the maternally and the paternally derived alleles. We conclude that the *ASCT2* escapes from imprinting in human placenta. Because imprinting patterns can vary in a developmental stage-specific and tissue-specific manner, evaluation of purified syncytiotrophoblasts at earlier phases of gestation may be required in future studies.

**Logistic regression reveals a tetragenic interaction underlying the mouse epistatic circler phenotype.** *E. Franssen*<sup>1</sup>, *K. Cryns*<sup>1</sup>, *F.L. Wuyts*<sup>2</sup>, *G. Van Camp*<sup>1</sup>. 1) Center for Medical Genetics, University of Antwerp (UIA), Antwerp, Antwerp, Belgium; 2) Department of ENT, University Hospital of Antwerp (UZA), Antwerp, Belgium.

The epistatic circler mouse is characterized by circling behavior, caused by a malformation of the horizontal semicircular canal (Cryns et al. unpublished results). This phenotype is observed in a fraction of the F2 generation from an intercross between *C57L/J* and *SWR/J* mouse strains. Genetic mapping studies have indicated that mutations in at least four different genes contribute to this condition: one gene from from the *SWR/J* strain (referred to as *Ecs*), localized at mouse chromosome 14, and three genes originating from the *C57L/J* strain (referred to as *Ecla*, *Eclb* and *Eclb*), located on chromosome 3, 4 and 13 respectively (Cryns et al. Genome Res. 12:613-617 (2002)). The recessive *Ecs* gene is necessary but not sufficient, whereas the exact nature of the contribution of the *Ecl* genes is less clear.

The F2 generation of the *C57L/J* X *SWR/J* intercross yielded 144 mice homozygous for the disease-associated *Ecs* allele carrying all possible combinations of *Ecl* alleles. Ninety-one mice were affected and 47 were not. Here we perform a logistic regression analysis to model the contribution of the three *Ecl* genes. Stepwise logistic regression indicates that the effect of these genes is non-additive. The *Eclb* locus is the main locus. *Ecla* and *Eclc* only contribute in the presence of at least one copy of *Eclb*.

This study proves that it is at least in model organisms possible to identify the genes involved in oligogenic traits. As vestibular dysfunction in man is probably a multigenic trait, identification of the genes involved in the epistatic circler mouse has considerable relevance to the study of balance disorders.

**SHFM3 is Associated with a Genomic Rearrangement in 10q24.** *X.J. de Mollerat<sup>1</sup>, D.B. Everman<sup>1</sup>, F. Gurrieri<sup>2</sup>, C.T. Morgan<sup>1</sup>, E. Sangiorgi<sup>2</sup>, P. Gaspari<sup>2</sup>, J. Amiel<sup>3</sup>, M.J. Bamshad<sup>4</sup>, R. Lyle<sup>5</sup>, J.E. Allanson<sup>6</sup>, B. Le Marec<sup>7</sup>, H. van Bokhoven<sup>8</sup>, N.E. Braverman<sup>9</sup>, U. Radhakrishna<sup>5</sup>, V. Elghouzzi<sup>3</sup>, S. Antonarakis<sup>5</sup>, R.E. Stevenson<sup>1</sup>, A. Munnich<sup>3</sup>, G. Neri<sup>2</sup>, C.E. Schwartz<sup>1</sup>.* 1) Greenwood Genetic Center, Greenwood, SC; 2) Istituto di Genetica Medica, Univ Cattolica, Roma, Italy; 3) INSERM U-393 Hopital Necker-Enfants Malades, Paris, France; 4) Dept of Genetics, Univ of Utah, Salt Lake City, UT; 5) Division of Medical Genetics, Univ of Geneva Medical School, Geneva, Switzerland; 6) Dept of Genetics, Childrens Hospital of Eastern Ontario Research Inst, Ontario, Canada; 7) Facult de Mdecine de Rennes I, Rennes, France; 8) Dept of Human Genetics, Univ Medical Centre Nijmegen, Nijmegen, The Netherlands; 9) John Hopkins Univ, School of Medicine, Baltimore, MD.

Split Hand-Split Foot Malformation (SHFM) is characterized by hypoplasia/aplasia of the central digits with fusion or deformation of the remaining digits. One locus for this condition, SHFM3, is located in 10q24. The phenotype of the Dactylaplasia (Dac) mouse, which has a SHFM-like phenotype, results from two different alleles, Dac1J and Dac2J. Both alleles arise from a disruption of the dactylin gene located in a region syntenic to 10q24. Mutation analysis of the DACTYLIN gene was conducted in 9 SHFM probands from families linked to 10q24. Screening of the coding region of DACTYLIN by SSCP and sequencing, failed to detect any point mutations. However, Southern, PFGE and dosage analyses demonstrated a complex rearrangement associated with a ~0.5 Mb tandem duplication in all the probands. Analysis of additional individuals found two individuals with non-familial SHFM with this rearrangement. The distal breakpoints are clustered in an 80 kb region and the proximal breakpoints are clustered in a 130 kb region. This duplicated region contained an extra copy of a portion of the DACTYLIN gene and the entire LBX1 and B-TRCP genes, both of which are known to be involved in limb development. Thus, it would appear that SHFM3 is a genomic disorder, a growing class of disorders associated with submicroscopic duplications, deletions or inversions.

**Polymorphisms in dysbindin (DTNBP1, 6p22.3) are associated with intermediate phenotypes measured by the Premorbid Adjustment Scale (PAS) in cases of childhood onset psychosis.** *M. Gornick<sup>1</sup>, A.M. Addington<sup>1</sup>, A. Sporn<sup>1</sup>, N. Gogtay<sup>1</sup>, D. Greenstein<sup>1</sup>, M. Lenane<sup>1</sup>, P. Gochman<sup>1</sup>, D.R. Weinberger<sup>2</sup>, J.L. Rapoport<sup>1</sup>, R.E. Straub<sup>2</sup>.* 1) Child Psychiatry Branch, NIH/NIMH, Bethesda, MD; 2) Clinical Brain Disorders Branch, NIMH, NIH, Bethesda, MD.

Straub et al (2002) recently identified 6p22.3 gene dysbindin as a schizophrenia susceptibility gene through a positional cloning effort. We studied a rare cohort consisting of 98 probands who have onset of psychotic symptoms before the age of 13. Seventy-one of these probands received a diagnosis of schizophrenia, and the remaining 27 have psychosis NOS (not otherwise specified), 11 of whom have subsequently developed bipolar disorder. Information on these subjects included cognitive and behavioral ratings of early development, history of medication response, neuropsychological and cognitive test performance, smooth pursuit eye movements, and premorbid development. We tested SNPs in and around dysbindin and performed family-based TDT analysis using the Spielman program TDT-STDT, haplotype analyses using the program TRANSMIT, and quantitative TDT using the software QTDT. A SNP allele located upstream of exon 1 was preferentially transmitted to affecteds (TDT-STDT p value 0.01). Furthermore, the QTDT analyses showed several significant findings. In particular, transmission of the rare alleles of 2 adjacent SNPs was strongly associated (empirical p values = 0.0009-0.003) with worse premorbid functioning as measured by the Premorbid Adjustment Scale (PAS). These findings strengthen the evidence that dysbindin is an important risk factor for schizophrenia and may play a key role in premorbid development. REFERENCES: Straub et al. Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia *AJHG* 71, 337 (2002).

**Haplotype structure of the UDP-glucuronosyltransferase 1A1 (*UGT1A1*) gene and its relationship to serum total bilirubin concentration in a Korean male population.** C.S. Ki<sup>1</sup>, K.A. Lee<sup>2</sup>, S.Y. Lee<sup>1</sup>, H.J. Kim<sup>1</sup>, S.S. Cho<sup>3</sup>, J.H. Park<sup>3</sup>, S. Cho<sup>3</sup>, K.M. Sohn<sup>3</sup>, J.W. Kim<sup>1</sup>. 1) Department of Laboratory Medicine, Samsung Medical Center, Seoul, Korea; 2) Department of Laboratory Medicine, College of Medicine, Korea University, Seoul, Korea; 3) Clinical Research Center, Samsung Biomedical Research Institute, Seoul, Korea.

Several polymorphisms in the UDP-glucuronosyltransferase 1A1 (*UGT1A1*) gene have been reported to be associated with the serum total bilirubin (T-Bil) concentration. Since these polymorphisms lie in a relatively short region, a certain extent of linkage disequilibrium (LD) is expected between them. We tried to investigate the haplotype structure of the *UGT1A1* gene and investigated its relationship to the serum T-Bil in a Korean male population. Three common polymorphisms in the *UGT1A1* gene were genotyped in 324 healthy Korean male subjects by direct sequencing; -3279T>G, (TA)<sub>6/7</sub> (*UGT1A1*\*28), and 211G>A (G71R; *UGT1A1*\*6). We assessed the extent of LD between these polymorphisms to construct multisite haplotypes and investigated the relationship between the *UGT1A1* haplotypes and the serum T-Bil concentration. There was significant pairwise LD ( $p < 0.0001$ ) with  $D'$  values of 1.0 between all pairs of the three polymorphisms. Haplotype analysis revealed that four out of eight possible haplotypes existed in the subjects; 3279T-(TA)<sub>6</sub>-211G, 3279T-(TA)<sub>6</sub>-211A, 3279G-(TA)<sub>6</sub>-211G, and 3279G-(TA)<sub>7</sub>-211G. When the subjects were stratified into 10 subgroups according to their *UGT1A1* haplotypes, significant differences in the mean serum T-Bil concentration were observed among the subgroups ( $p=0.0001$ ). In conclusion, we demonstrated that there is complete LD between three common polymorphisms in the *UGT1A1* gene. Furthermore, we unequivocally constructed the haplotypes of the *UGT1A1* gene, and revealed that they were significantly associated with the serum T-Bil concentration in a Korean male population. To the best of our knowledge, this is one of the first studies to assess the haplotype-phenotype correlation between the *UGT1A1* gene and the serum T-Bil concentration.

**Glucocerebrosidase Mutations in Subjects with Parkinson Disease.** A.A. Lwin<sup>1,2</sup>, E. Orvisky<sup>1,2</sup>, M. Eblan<sup>1,2</sup>, E. Sidransky<sup>1,2</sup>. 1) Section on Molecular Neurogenetics, NIMH, NIH, Bethesda, MD; 2) Medical Genetics Branch, NHGRI, NIH, Bethesda, MD.

Recent studies suggest an association between Gaucher disease, the inherited deficiency of glucocerebrosidase, and parkinsonism. A small subset of patients with Gaucher disease who develop an early-onset, treatment refractory form of parkinsonism have been reported. Upon recent inspection, it has been noted that some Gaucher probands also have close relatives with Parkinson disease. In order to determine whether glucocerebrosidase may contribute to the etiology of parkinsonism in other cases, we determined the glucocerebrosidase gene (GBA) sequence and enzyme activity in 57 samples of brain tissue from subjects with a primary diagnosis of Parkinson disease and in tissue from 47 controls obtained from four different brain banks. Alterations in the glucocerebrosidase gene were identified in 12 samples (21%), including eight with functional mutations in GBA (N370S, L444P, K198T, and R329C) and four with probable polymorphisms (T369M and E326K). Two subjects belonged to a subset of 29 individuals who died before age 76. Thus, among these younger subjects with Parkinson disease, 27% were Gaucher carriers or homozygotes. The only GBA alterations identified among the 47 control samples were two alleles with E326K. These results suggest that mutations in glucocerebrosidase may be associated with the development of parkinsonism and altered glucocerebrosidase, even in heterozygotes, may be a risk factor for parkinsonism in some individuals.

**Chromosomal loci of alleles that protect the mouse retina from light-induced damage.** *M. Danciger*<sup>1</sup>, *J.E. Lyon*<sup>1</sup>, *D.M. Worrill*<sup>1</sup>, *J. Lem*<sup>2</sup>, *C. Grimm*<sup>3</sup>, *A. Wenzel*<sup>3</sup>, *C.E. Remé*<sup>3</sup>. 1) Dept Biol, Loyola Marymount Univ, Los Angeles, CA; 2) Ophthalmology & Cardiology, Tufts-New England Med Ctr, Boston, MA; 3) 3Laboratory of Retinal Cell Biology, University Hospital Zurich, Zurich, Switzerland.

Retinas of BALB/cByJ mice are more susceptible to light insult than those of 129S1/SvImJ. In this work, we used quantitative genetics to determine the loci that influence intense light-induced retinal damage as a step toward identifying the genes/alleles that the loci represent. Thus, 289 4-6 week old F2 progeny of an intercross between 129 and BALB/c mice were exposed to 15,000 LUX for 1 hour after pupil dilation, kept in the dark for 16 hours and then placed in dim cyclic light for 10-12 days. At the end of this time, the amount of rhodopsin remaining in the retinas was measured spectrophotometrically and was used as the quantitative trait measuring retinal damage. Neither gender nor pigmentation had a significant influence on rhodopsin loss. DNAs from F2 progeny were genotyped with 72 dinucleotide repeat markers spanning the genome. For screening purposes, the markers were first tested in 27-36 of the F2 progeny with the most rhodopsin remaining and 27-36 with the least rhodopsin. Any marker with a >95% probability of being associated with phenotype was tested in all F2s. Additional markers were typed in significant regions. Data were analyzed with the Map Manager QTX program. QTL on mouse Chrs 1 and 4 were identified with LOD scores of 6.8 and 7.0 and with percent genetic effects of 28% and 28%, respectively. In addition, two suggestive QTL were found on Chrs 6 and 2 with LOD scores of 3.6 and 3.1 each accounting for 14% of the genetic effect. For each of the QTL the 129 alleles protect the retina from light damage, and the four QTL add up to 84% of the total genetic effect. The Chr 1 QTL is in the same region as a QTL influencing constant light-induced retinal damage between two other mouse strains, and the Chr 6 QTL is in the same region as a QTL influencing age-related retinal degeneration between the BALB/cByJ and C57BL/6J-c2J strains. Identification of the gene modifiers represented by these QTL may be important for human retinal disorders that are accelerated by light exposure.

**Refined mapping of the DYX3 dyslexia susceptibility region.** *H. Anthoni<sup>1</sup>, M. Peyrard-Janvid<sup>1</sup>, P. Onkamo<sup>1</sup>, N. Kaminen<sup>2</sup>, K. Hannula<sup>2</sup>, J. Nopola-Hemmi<sup>3</sup>, H. Lyytinen<sup>4</sup>, J. Kere<sup>1, 2</sup>.* 1) Dept. of Biosciences at NOVUM, Karolinska Institute, Huddinge, Sweden; 2) Dept. of Medical Genetics, Biomedicum, University of Helsinki, Finland; 3) Dept. of Pediatric Neurology, Hospital for Children and Adolescents, University of Helsinki, Finland; 4) Dept. of Psychology and Child Research Center, University of Jyväskylä, Finland.

Developmental dyslexia is a complex disease characterized by unexpected difficulty in learning to read despite adequate intelligence, education, and normal senses. It has a prevalence of 5-15% and a wide spectrum of phenotypes probably due to the effects of multiple genes, incomplete penetrance and environmental factors. Several genetic loci have been mapped to at least five chromosomes, but to date no gene has been reported to be involved in the development of dyslexia. Our genome-wide scan results from a set of eleven Finnish families pointed out a 40 cM region located 30 cM centromeric to the previously reported dyslexia locus DYX3 on 2p16-p15. Non-parametric linkage (NPL) analysis showed the highest score of 2.55 ( $p=0.004$ ) for marker D2S2216 at 2p11.2. We performed two rounds of fine mapping using 29 additional microsatellite markers in the same set of families, as well as in 64 Finnish case-controls. These gave the highest NPL score of 3.014 ( $p=0.001$ ) for marker D2S2216. Association analysis using Haplotype Pattern Mining (HPM) gave the highest score for marker D2S286 ( $p<0.001$ ) in the six pedigrees showing linkage to the region. Together, these data narrow down the candidate region to 8 cM on 2p12-p11.2 and makes it distinct from the DYX3 locus. We have tested two genes, TACR1 and CTNNA2, for mutations in affected individuals from our set of families. These genes made good candidates as they were located within the linked region and are involved in some functions of the nervous system. No mutations could be detected in the coding regions of these two genes. To further narrow down our candidate region, we are presently adding single nucleotide polymorphism (SNP) markers to this region, and proceeding with the positional cloning of the gene.

**Evidence for gene-gene interaction in susceptibility to bipolar disorder.** *A.M. Addington<sup>1,3</sup>, C.D. Langefeld<sup>2</sup>, P.P. Zandi<sup>3</sup>, J.R. DePaulo<sup>3</sup>, J. Nurnberger<sup>4</sup>, T. Reich<sup>5</sup>, E. Gershon<sup>6</sup>, M.G. McInnis<sup>3</sup>.* 1) Child Psychiatry Branch, NIH/NIMH, Bethesda, MD; 2) Dept of Public Health Sciences, Wake Forest Univ Sch Med, Winston-Salem, NC; 3) Dept Psychiatry, Johns Hopkins Univ Sch Med, Baltimore, MD; 4) Indiana University, Indianapolis, IN; 5) Washington University, St. Louis, MO; 6) University of Chicago, Chicago, IL.

We performed a nonparametric linkage analysis of genome scan data from 148 multiplex bipolar pedigrees from the Hopkins/DANA family study and Wave 1 of the NIMH Genetics Initiative. Using a narrow phenotype model, including BPI, BPII and SA/BP as affected (assessed by SADS-L/RDC or DIGS), both single-locus and multi-locus nonparametric linkage analyses were completed. The NPL regression approach (Langefeld, 1999) utilizes the multipoint pedigree-specific NPL scores derived from the program GENEHUNTER as covariates in a conditional logistic regression model that tests for increased allele sharing. The regression model allows for joint and conditional tests of multiple loci, including evidence for heterogeneity and epistasis. The subset approach (Cox et al., 1999) weights families on evidence for linkage at one trait locus in order to test for linkage at other unlinked regions of the genome. Single-locus analyses of this combined dataset revealed suggestive evidence for linkage on chromosome 2q near D2S160 (LOD = 2.3) and 8q near D8S256 (LOD = 2.3). A multi-locus model using the NPL regression method increased evidence for linkage to 8q to a LOD of 3.8, adjusting for linkage evidence at five other regions. Both approaches identified a putative trait locus on chromosome 5q near D5S400 that did not show evidence for linkage in single-locus analysis, suggesting a significant epistatic interaction with the locus on chromosomes 2q ( $p = 0.000017$ ). Statistical approaches that are able to test for linkage at multiple regions simultaneously may provide more power as they more accurately reflect the underlying biology of complex traits and genetic heterogeneity present in collected samples.

**The metabolic clearance rate of insulin: a novel heritable risk factor for atherosclerosis.** *M.O. Goodarzi<sup>1,2</sup>, M.J. Quiñones<sup>2</sup>, H. Yang<sup>1</sup>, X. Li<sup>1</sup>, D. Wang<sup>1</sup>, I. Enriquez<sup>2</sup>, X. Jimenez<sup>2</sup>, G. Hernandez<sup>2</sup>, R. De La Rosa<sup>2</sup>, Y. Li<sup>3</sup>, M.F. Saad<sup>2</sup>, H.N. Hodis<sup>3</sup>, W.A. Hsueh<sup>2</sup>, J.I. Rotter<sup>1,2</sup>.* 1) Cedars-Sinai Medical Center, Los Angeles, CA; 2) UCLA, Los Angeles, CA; 3) USC, Los Angeles, CA.

The goal of the Mexican-American Coronary Artery Disease (MA-CAD) project is to identify genes linking insulin resistance and atherosclerosis. 101 MA families were recruited via a parent with CAD; all adults (age 18, n=568) were genotyped for 408 microsatellite markers along the genome at ~10cM density. Adult offspring and offspring spouses (n=438) underwent the euglycemic clamp and ultrasound assessment of carotid intima-media thickness (IMT), a measure of subclinical atherosclerosis. In addition to directly quantifying insulin sensitivity (whole body glucose disposal, M), the clamp yields a measurement of the metabolic clearance rate of insulin (MCRI). MCRI was correlated with IMT ( $r=0.15$ ,  $P=0.003$ ), as was M ( $r=-0.013$ ,  $P=0.009$ ). After adjusting for age, gender, and BMI, MCRI was still significantly associated with IMT ( $p=0.028$ ) while M was not ( $p=0.71$ ). Heritability estimates and multipoint linkage analyses were carried out with variance component methods in SOLAR. MCRI was found to have a heritability ( $h^2$ ) of 0.83 ( $P0.001$ ); of note, this was higher than the heritability of M ( $h^2=0.40$ ,  $P0.001$ ). A genome-wide linkage scan revealed several peaks for MCRI on chromosomes 1 (124 cM, LOD 1.85), 4 (105 cM, LOD 1.56), 6 (57 cM, LOD 1.98), 8 (37 cM, LOD 1.40), and 9 (139 cM, LOD 2.55). Insulin binding to its receptor triggers internalization of the insulin-receptor complex, which leads to clearance of insulin. Thus, MCRI may be a marker of insulin binding and action. The positive association between MCRI and IMT suggests that increased insulin action may lead to subclinical atherosclerosis, most likely via the mitogenic/growth promoting effects of insulin. We also show that MCRI is highly heritable and report several loci exhibiting linkage with MCRI. MCRI appears to be a previously unrecognized risk factor for atherosclerosis. Identification of gene(s) that influence MCRI will provide further insight into the connection between insulin action and atherosclerosis underlying the metabolic syndrome.

**Polymorphisms in the CTLA4 gene region confer susceptibility to Addisons disease.** A. Blomhoff<sup>1</sup>, B.A. Lie<sup>2</sup>, E.H. Kemp<sup>3</sup>, A.P. Weetman<sup>3</sup>, H.E. Akselsen<sup>1</sup>, A.G. Myhre<sup>4</sup>, E.S. Husebye<sup>5</sup>, D.E. Undlien<sup>1</sup>. 1) Institute of Medical Genetics, Ullevål University Hospital, University of Oslo, Norway; 2) Institute of Immunology, Rikshospitalet University Hospital, Oslo, Norway; 3) Division of Clinical Science (North), University of Sheffield, United Kingdom; 4) Department of Pediatrics and Department of Clinical Molecular Biology, Akershus University Hospital, Norway; 5) Medical Department B, Haukeland University Hospital, University of Bergen, Norway.

Addison's disease (primary adrenal insufficiency), is a rare disease with a prevalence of 4-14/100 000 in Caucasians, and is mainly caused by an autoimmune adrenalitis. The disease may occur isolated or as a part of an autoimmune polyendocrine syndrome (APSI or APSII). While APSI is a monogenic disease, both APSII and isolated autoimmune Addisons disease are genetically complex disorders. The CTLA4 (cytotoxic T lymphocyte antigen4) gene on chromosome 2q33 is associated with several autoimmune disorders. Studies on autoimmune Addisons disease and polymorphisms in the CTLA4 gene have been inconclusive. The gene encodes the CTLA4 surface molecule on activated T- lymphocytes, involved in down regulation of the immune response. New candidate polymorphisms located within a 6.1 kb region downstream of the gene have recently been revealed to be strongly associated with disease susceptibility for Graves' disease, type 1 diabetes and Hashimotos thyroiditis. These SNPs (single nucleotide polymorphisms) are probably also influencing gene splicing, and thereby the relative abundance of soluble CTLA4 versus full length isoforms. We have genotyped a total of 134 Addison's disease patients from Norway and UK with either isolated autoimmune Addison's disease or APSII, and 414 healthy controls for 5 of these newly identified SNPs. We find that alleles at these SNPs differ significantly in Addison's disease patients compared to controls. Our results demonstrate that the same polymorphisms that confer susceptibility to the above mentioned autoimmune diseases also confer susceptibility to Addison's disease. This supports that CTLA4 polymorphisms may confer susceptibility to autoimmunity in general.

Program Nr: 2121 from 2003 ASHG Annual Meeting

**A SNP in NCAM1 shows association with human neural tube defects.** *K.L. Bastress, E.C. Melvin, D. Siegel, J.R. Gilbert, D.S. Enterline, T.M. George, M.C. Speer, NTD Collaborative Group.* Duke University Medical Center, Durham, NC.

Neural tube defects (NTDs) are common birth defects with an incidence of approximately 1/1000 births with both genetic and environmental factors implicated. To date, no major genetic factors related to risk have been identified. Throughout development, cell adhesion molecules are strongly implicated in cell-cell interactions, and may play a role in the formation and closure of the neural tube. There has been evidence that expression of cell adhesion molecules are disturbed in spontaneous NTDs in avian embryos. In order to test whether neural cell adhesion molecule 1 (NCAM1) influences risk of human NTDs, we evaluated single nucleotide polymorphisms (SNPs) within the gene. Four SNPs within the NCAM1 were genotyped using TaqMan: rs2298526, rs2011505, ims\_jst013324, and rs1006826. We utilized both family-based and case-control approaches to evaluate evidence for association and/or linkage disequilibrium in this data series. We analyzed our sample set (N = 172 families) of American Caucasian simplex lumbosacral myelomeningocele families and 252 unrelated controls for evidence of linkage and association using TRANSMIT and the pedigree disequilibrium test (PDT). Transmit analysis revealed a significant association between risk for NTDs and intronic SNP rs2298526 ( $p = 0.0028$ ). Using both the PDT and geno-PDT, evidence for significant association was again found in SNP rs2298526 ( $p = 0.042$ ). These results suggest variations in NCAM1 may influence risk for human NTDs.

**Analysis of candidate genes for nuclear-encoded modifiers in families with the mitochondrial A1555G mutation and non-syndromic deafness.** *Y. Bykhovskaya<sup>1</sup>, E. Mengesha<sup>1</sup>, D. Wang<sup>1</sup>, H. Yang<sup>1</sup>, M. Shohat<sup>2</sup>, X. Estivill<sup>3</sup>, N. Fischel-Ghodsian<sup>1</sup>.*

1) Ahmanson Department of Pediatrics, Steven Spielberg Pediatric Research Center, Medical Genetics Birth Defects Center, Cedars-Sinai Medical Center and UCLA School of Medicine, Los Angeles, CA; 2) Department of Pediatrics and Medical Genetics, Basil and Gerald Felsenstein Medical Research Center, Tel Aviv University Medical School, Petah Tikva, Israel; 3) Deafness Genetics Research Group, Medical and Molecular Genetics Center, Hospital Duran I Reynals, Barcelona, Spain.

Phenotypic expression of the deafness-associated mitochondrial A1555G mutation in the 12S rRNA gene is influenced by aminoglycosides and complex inheritance of nuclear-encoded modifier genes. The position of a major modifier gene has been localized to 8p23.1, but the locations of other modifiers remain unknown. Genes for prothocadherin 15 (PCDH15), cadherin 23 (CDH23), homolog of yeast mto1 gene (MTO1), homolog of yeast mss1 gene (GTPBG3), mitochondrial transcription factors B1 (TFB1M) and B2 (TFB2M) have been proposed as candidates for being modifiers. We tested a total of 214 DNA samples from 27 pedigrees of Spanish and Italian background, and from a large Arab-Israeli kindred with maternally inherited deafness for linkage and association using SNPs and microsatellites in these genes. The non-parametric linkage analysis and TDT testing were done using all families combined as well as in the dataset divided based on linkage to the chr 8 locus and ethnicity. MTO1 and TFB1M showed strongly suggestive linkage and LD results in all families combined thus suggesting their function as nuclear-encoded modifier genes for the A1555G mutation. Sequencing analysis of the coding sequence and exon-intron junctions of the TFB1M and MTO1 genes in a subset of affected and unaffected Arab-Israeli, Spanish, and Italian family members did not reveal any relevant sequence variants. Understanding the regulation of transcription and splicing of TFB1M and MTO1, and sequencing of the regulatory regions will help clarify their involvement in the pathogenesis of maternally inherited deafness, and possibly in other mitochondrial DNA disorders. We gratefully acknowledge support by NIH/NIDCD grant RO1DC01402.

**Significant evidence for a *CARD15*-independent Crohns Disease susceptibility locus on chromosome 16.** *K.E. Adams, N.J. O'Callaghan, J.A. Cavanaugh.* Medical Genetics Research Unit, JCSMR - The Canberra Hospital, Woden, Australia.

Inflammatory bowel disease (IBD) is a complex genetic disease comprising the disease subsets of Crohns disease (CD) and ulcerative colitis (UC). Susceptibility loci for IBD have been implicated on a number of chromosomes, with the most replicated and validated being *IBD1* on chromosome 16q and *IBD3* on chromosome 6p. Three SNPs in *CARD15* have been implicated at *IBD1*, while a SNP in the promoter of *TNF*- (-857<sub>CT</sub>) has been implicated at *IBD3*. Hampe et al (2003) have recently suggested that there is a second IBD susceptibility locus on the p arm of chromosome 16, designated *IBD8*.

To investigate the possibility of a second locus we analysed a dense microsatellite marker map of the p arm of chromosome 16 in 75 Australian CD families. Maximal two point linkage (NPL=3.0) is observed at D16S685 which is located at 16p11, more than 19cM from *CARD15*. Multipoint analysis also shows significant evidence for linkage in the region of D16S685 and D16S753. TDT provides further support (p=0.002) for this locus, particularly at D16S3145, the marker previously implicated by Hampe et al (2003). Conditional analyses shows that linkage in this region is strongest in those families in which the *TNF*- -857T allele is segregating (NPL=4.0).

We have previously reported strong evidence for linkage between *IBD1* and CD in the Australian population, as well as association between two of the three risk alleles reported in *CARD15* and CD. We have also reported strong evidence for an association between the *TNF*- -857C allele and *CARD15*. Our data supports the presence of an independent locus at *IBD8*, through linkage and TDT. In addition, the association with the *TNF*- -857T allele, rather than the C allele, suggests that this novel locus is independent of *CARD15*.

**Differential allelic expression of BMP5 and COL9A1 may constitute the osteoarthritis susceptibility that has been mapped to chromosome 6.** *A. Ferreira, L. Southam, B. Dowling, L. Marcelline, Z. Mustafa, K. Chapman, G. Bentham, J. Loughlin.* Botnar Research Centre, University of Oxford, Oxford.

Primary osteoarthritis (OA) is a common disease characterised by the degeneration of the cartilage of articulating joints. We have previously linkage mapped a hip OA susceptibility locus to a 12cM interval on chromosome 6, with a maximum multipoint LOD score of 4.0. This linkage was restricted to the 146 families from our cohort that contained female affected sibling pairs concordant for hip OA. As the first stage in the association mapping of this locus, we have genotyped 36 microsatellite markers from within the 12 cM interval in the 146 female-hip families. The highest two-point LOD score was 4.8, with 11 markers having LOD scores  $> 2.0$ . We tested each marker for association to OA by the TDT. We also genotyped 220 age-matched female controls and performed a case-control analysis, using the 146 probands from our families as the cases. These tests provided moderate evidence ( $P < 0.05$ ) for association to microsatellites located close to two candidate genes: the bone morphogenetic protein 5 gene (BMP5) and COL9A1, which encodes for the 1 polypeptide chain of type IX collagen. BMP5 is a regulator of chondrocyte differentiation whilst type IX collagen is a structural component of the cartilage extracellular matrix. We had previously failed to detect association to these candidates using gene-based SNPs. Our latest data prompted us to speculate that the chromosome 6 OA susceptibility could be coded for by cis-acting variation in the regulatory elements of one or both of these two genes rather than by an alteration in protein sequence. To test this we extracted RNA from the articular cartilage of OA patients heterozygous for coding SNPs within BMP5 or COL9A1 and compared allelic output by rtPCR and single base extension. 30% of our patients demonstrated differential allelic expression at BMP5, 50% at COL9A1. This analysis implies that variation in the level of expression of these two candidates may constitute the OA susceptibility that we have linkage mapped to chromosome 6. We need to identify the cis-acting sequences responsible for this variation to be certain of this.

**High-Density Lipoprotein Cholesterol Locus on Chromosome 16 Restricted to 3.5 Mb.** *A.G. Day<sup>1</sup>, E. Sobel<sup>1</sup>, K. Lange<sup>1</sup>, A.J. Lusis<sup>1</sup>, T.W.A. de Bruin<sup>2</sup>, M.R. Taskinen<sup>3</sup>, P. Pajukanta<sup>1</sup>.* 1) Human Genetics, UCLA, Los Angeles, CA; 2) Maastricht University, The Netherlands; 3) Helesinki University Central Hospital, Finland.

Coronary heart disease (CHD) is a multifactorial disease constituting a major health problem in Western societies. We aim to assess the largely unknown genetic background of CHD by investigating the two most common dyslipidemias predisposing to CHD, familial combined hyperlipidemia (FCHL) and low serum high-density lipoprotein cholesterol (HDL-C). These two disorders taken together affect 20-40% of families with premature CHD. We recently identified a 25-cM susceptibility locus for HDL-C on chromosome 16 in a combined analysis of Finnish and Dutch genome scans. The current aim is to restrict this 25-cM region via an analysis strategy incorporating genotyping error and locus heterogeneity considerations. Linkage analysis is sensitive to genotyping errors and locus heterogeneity in the study sample, of which both introduce noise and increase the difficulty of finding the true signal. We employed a refinement strategy to the 25-cM region on 16q that minimizes these effects. The strategy included: (1) Zeroing out all genotypes that have a posterior probability of mistyping at either allele greater or equal to an heuristically determined value of 0.5; (2) Estimating the alpha value of the cleaned data by linkage analysis; (3) Utilizing the estimated alpha value (0.5) to calculate posterior probability of linkage for each family; and finally (4) Perform linkage analysis only on those families with posterior probability greater or equal to an heuristically determined value of 0.6. This strategy refined the originally 25-cM region to a 9-cM region (i.e. the Lod minus 1 region) on chromosome 16q. Importantly, this 9-cM region equals to only 3.5-Mb with completely finished sequence and involves 40 genes, thus providing a considerably restricted region to target for fine mapping with the goal of identifying the associated gene.

**CTLA4 is a susceptibility gene for Systemic Lupus Erythematosus.** *M. Barreto*<sup>1</sup>, *E. Santos*<sup>3</sup>, *R. Ferreira*<sup>1</sup>, *C. Fesel*<sup>1</sup>, *F. Fontes*<sup>1</sup>, *C. Pereira*<sup>2</sup>, *B. Martins*<sup>2</sup>, *R. Andreia*<sup>3</sup>, *J. Viana*<sup>3</sup>, *F. Crespo*<sup>3</sup>, *C. Vasconcelos*<sup>3</sup>, *C. Ferreira*<sup>3</sup>, *A.M. Vicente*<sup>1</sup>. 1) Instituto Gulbenkian Ciência, Oeiras, Portugal; 2) Instituto de Ciências Biomedicas Abel Salazar, Porto, Portugal; 3) Associação dos Doentes com Lupus, Portugal.

Systemic Lupus Erythematosus (SLE) is a genetically complex autoimmune disorder of unknown etiology. Dysregulation of the co-stimulatory system likely contributes to the initiation and maintenance of autoimmunity due to activation of self-reactive T cells. The CTLA4 molecule, which has a role in down-regulating the activation of T cells, has been implicated in a number of autoimmune diseases such as IDDM and Multiple Sclerosis. To investigate the role of CTLA4 in the pathogenesis of SLE, we genotyped two polymorphic markers within the CTLA4 gene (an A to G transition in exon 1 and a microsatellite in the 3' UTR), in a Portuguese population sample of 115 patients and 176 controls. We found a significant association with the CTLA4 microsatellite marker ( $\chi^2=24.190$ ,  $p=0.001$ ) with a particular allele showing a protective effect ( $\chi^2=17.310$ ,  $p=0.001$ ) and another conferring susceptibility to SLE ( $\chi^2=4.636$ ,  $p=0.031$ ). Genotypes containing either the protective or the susceptibility allele were associated with SLE ( $\chi^2=16.857$ ,  $p<0.001$  and  $\chi^2=6.430$ ,  $p=0.011$ , respectively). Although the male:female ratio is not equal in the case and control samples, matching for sex did not change the overall association ( $\chi^2=21.247$ ,  $p=0.003$ ). Because case-control studies are prone to errors due to population admixture, we ran FBAT on a sample of 55 multiplex families (comprising 73 patients). We found a transmission disequilibrium of the susceptibility allele (more often transmitted to affected offspring,  $p=0.01$ ), thus corroborating the association result obtained in the case-control study. No association was found with the SNP on CTLA4 exon 1. Because the 3' UTR polymorphism may be involved in the regulation of CTLA4 mRNA stability and, consequently, of its expression, we propose that the genotype-dependent defective expression of this molecule may be responsible for a deficient down-regulation of the immune response, leading to autoimmunity in SLE.

**Multigenic obesity congenic strains as novel models for cardiovascular complications of diabetes.** *D. Estrada-Smith<sup>1</sup>, X. Wang<sup>2</sup>, P. Wen<sup>2</sup>, L.W. Castellani<sup>2</sup>, A.J. Lusis<sup>1, 2</sup>, R.C. Davis<sup>2</sup>.* 1) Human Genetics, UCLA School of Medicine, Los Angeles, CA 90095; 2) Cardiology, UCLA School of Medicine, Los Angeles CA 90095.

Multigenic obesity (MOB) loci 5 and 6 influence interrelated phenotypes of obesity, insulin resistance and dyslipidemia as demonstrated by recent characterization of MOB congenic strains. Here a genomic interval from 63-165 Mb (middle MOB) from the lean CAST/Ei (CAST) strain introgressed onto an obesity susceptible C57BL/6 (BL6) background produced a congenic mouse strain displaying an atherogenic lipid profile (low HDL, high LDL and total cholesterol) that was resistant to obesity and maintained lower insulin and glucose levels compared to BL6 controls when fed an atherogenic or a high energy diet. Conversely mice with an introgressed genomic interval from 83-120Mb (distal MOB) from CAST showed multiple metabolic syndrome traits including increased plasma insulin, glucose, free fatty acids, triglycerides and adiposity when compared to controls. To further evaluate contributions of this locus to the development of atherosclerosis both congenic strains were bred onto an LDLR<sup>-/-</sup> background and fed a high fat diet. Recent NMR data indicate that middle MOB derived strains still continue to display a decrease in fat accretion when compared to distal MOB derived and LDLR<sup>-/-</sup> control strains. Similarly, this strain continues to show no impairment of glucose sensing as determined by IPGTT. And while middle MOB derived strains show little to no atherosclerotic lesion formation a subset of middle MOB mice show extensive calcification of the myocardium. In contrast, distal MOB derived strains demonstrate impaired glucose tolerance, increased adiposity and extensive atherosclerosis but no calcification. Much of the MOB locus is syntenic human loci associated with obesity, insulin resistance. Identification the susceptibility and resistance genes within the MOB loci and characterization of the pathways associated with them will reveal important interactions to elucidate the associations between the dyslipidemia, obesity and vascular disease associated with type 2 diabetes in humans.

***INSR* and *CACNA1A* are not Major Contributing Loci in Finnish Migraine Families.** M.A Kaunisto<sup>1</sup>, P.J. Tikka<sup>1,2</sup>, M. Kallela<sup>3</sup>, J.C. Papp<sup>4</sup>, A. Korhonen<sup>4</sup>, E. Hämäläinen<sup>1</sup>, T. Moilanen<sup>1</sup>, H. Harno<sup>3</sup>, H. Havanka<sup>5</sup>, M. Färkkilä<sup>4</sup>, R.A. Ophoff<sup>4</sup>, A. Palotie<sup>1,2,4,6</sup>, M. Wessman<sup>1</sup>. 1) Dept of Clinical Chemistry, Univ of Helsinki, Finland; 2) Finnish Genome Center, Univ of Helsinki, Finland; 3) Dept of Neurology, Univ of Helsinki, Finland; 4) Dept of Human Genetics, David Geffen School of Medicine, UCLA, USA; 5) Länsi-Pohja Central Hospital, Kemi, Finland; 6) Dept of Pathology, David Geffen School of Medicine at UCLA, USA.

Migraine is a common, complex neurological disorder characterized by severe unilateral pulsating headache with possible transient neurological aura symptoms. Mutations in the *CACNA1A* gene on 19p13, coding for the calcium channel alpha 1A subunit, have been demonstrated to cause familial hemiplegic migraine (FHM1), a rare autosomal dominant subtype of migraine with aura. Its contribution to the more common forms of migraine, migraine with or without aura, has been debated. The insulin receptor gene (*INSR*) on the 19p13 region has also been associated with migraine. To test the possible involvement of *INSR* and *CACNA1A* loci in migraine with aura (MA), we studied a 33 cM region covering these two loci by genotyping eight microsatellite markers: D19S247, D19S427, D19S592, D19S391, D19S394, D19S221, D19S1150, D19S226 in 72 Finnish, MA families with 757 individuals. The genotypes were analysed by the two-point parametric linkage analysis using the affecteds only model. Having MA patients as affected (n=416), the maximum combined lod scores for these markers were all 0.3 both under locus homogeneity and heterogeneity. Thus our results show no evidence for linkage to this region. When the family-based lod scores were examined, one family showed nominal linkage (lod score 1.0) to the *INSR* flanking marker D19S427 and one family to the *CACNA1A* flanking marker D19S226. Additionally, the marker D19S394 locating between the *INSR* and *CACNA1A* genes gave a lod score 1.0 in one family. Inspection of the haplotypes for each family did not support linkage. In conclusion, our findings do not support the previous studies that *INSR* and *CACNA1A* are susceptibility genes to MA. However, we can not rule out the possibility that the 19p13 region might contain a modifying locus to MA.

**Haplotype association studies in target regions on chromosomes 6q23-q27 and 14q21-q23 in systemic lupus erythematosus.** *C.M. Lindgren*<sup>1,3</sup>, *S. Koskenmies*<sup>2</sup>, *E. Widen*<sup>2</sup>, *P. Onkamo*<sup>2</sup>, *P. Sevnó*<sup>2</sup>, *V. Mkelää*<sup>1</sup>, *L. Berglind*<sup>1</sup>, *V. Ollikanen*<sup>2</sup>, *H. Julkunen*<sup>2</sup>, *J. Kere*<sup>1,3</sup>. 1) Dept. of Biosciences, Novum, Stockholm, Sweden; 2) Department of Medical Genetics, University of Helsinki, Finland; 3) Centre for Biotechnology at Novum, Karolinska Institutet, Stockholm, Sweden.

Systemic lupus erythematosus (SLE) is a chronic, multifactorial, autoimmune disease of unknown aetiology, which displays a broad variety of clinical manifestations. Epidemiological studies as well as recent genome wide scans (GWS) and studies of candidate genes have showed a genetic background of the disease and suggested several genetic loci that might be of importance for disease susceptibility. However, most of the genetic components of SLE are still unknown. We have previously conducted a GWS in 35 Finnish multiplex families containing 169 individuals (73 affected with SLE). Using 417 microsatellites, we detected suggestive linkage on chromosome 6q23-q27 (NPL 2.5,  $p=0.01$ ) and 14q21-q23 (NPL 2.2,  $p=0.02$ ) as well as in the HLA region on chromosome 6p. Here we have performed initial finemapping adding 44 microsatellite markers at 1 cM density in the chromosome 6q23-q27 (19 markers) and 14q21-q23 (25 markers) regions in trios from 65 families, including the individuals from the initial scan. The data was analysed using two complementary approaches, haplotype pattern mining (HPM) and transmission disequilibrium test (TDT) and all results were permuted to assess the empirical significance. Our result show an excess sharing of a haplotype on 14q21-q23 ( $p=0.006$ ) and excess transmission of a haplotype on 6q23-q27 ( $p=0.03$ ), adding strength to our hypothesis that these regions may contain susceptibility genes for SLE. Further finemapping at a 200 kb density using single nucleotide polymorphisms in these regions is currently undertaken.

**Association of APOC3 and APOA5 with Triglycerides and LDL Particle Size in Families with Familial Combined Hyperlipidemia.** *R. Mar<sup>1</sup>, P. Pajukanta<sup>1</sup>, H. Allayee<sup>1</sup>, R. Cantor<sup>1</sup>, J.S. Sinsheimer<sup>1</sup>, M. Groenendijk<sup>2</sup>, G. Dallingae-Thie<sup>2</sup>, T.W.A. de Bruin<sup>3</sup>, A.J. Lusis<sup>1</sup>.* 1) Human Genetics, UCLA, Los Angeles, Los Angeles, CA; 2) Vascular Medicine, University Medical Center Utrecht, Utrecht, the Netherlands; 3) Dept. of Medicine, Academic Hospital, Maastricht, the Netherlands.

Familial combined hyperlipidemia (FCH) is a complex lipid disorder characterized by elevated plasma triglyceride and cholesterol levels. The (APO)LIPOPROTEIN A1/C3/A4 gene cluster on chromosome 11 has long been recognized as a modifier of plasma triglycerides. APOA5 lies 27kb downstream of the cluster and has been shown to influence triglyceride levels in mice and humans. Previously, three restriction enzyme polymorphisms in the APOA1/C3/A4 gene cluster were analyzed in 18 Dutch FCH probands and their relatives. The rare alleles of Xmn and Msp sites in APOA1 and Sst site in APOC3 were more frequent in probands than in normolipidemic spouses. A specific combination of haplotypes with one chromosome carrying the X1M1S2 (1-1-2) haplotype and the other carrying the X2M2S1 (2-2-1) haplotype was associated with elevated plasma lipid levels when compared to the wild type combination of haplotypes (1-1-1/1-1-1). In this study, we extended the analysis to include APOA5 in order to study its effects on plasma triglycerides in FCH subjects. Seven SNPs across the APOA1/C3/A4/A5 region were analyzed in 80 FCH probands and their normolipidemic spouses and in 27 Dutch FCH families. In the case-control study, the strongest evidence of association was obtained with the Xmn and Msp SNPs in APOA1 ( $P = 0.0009$ ). Family based tests indicate significant association of triglycerides and LDL particle size with SNPs in APOC3 and APOA5. Two haplotypes, one containing the rare alleles of Xmn and Msp (2-2-1-1-1) and the other containing the rare alleles of Sst and a SNP within APOA5 (1-1-2-1-2) were enriched in probands when compared to normolipidemic spouses. A third haplotype containing the rare allele of a SNP within APOA4 (1-1-1-2-1) was over-represented in spouses. These data demonstrate a complex genetic contribution of this locus to FCH by conferring both susceptibility and resistance.

**Defining the linkage disequilibrium region containing the Tau gene and identifying haplotype tagging**

**polymorphisms.** *S.A. Oliveira<sup>1</sup>, E.R. Martin<sup>1</sup>, W.K. Scott<sup>1, 3</sup>, F. Zhang<sup>1</sup>, J.M. Stajich<sup>1</sup>, L.T. Middleton<sup>2, 3</sup>, A.D. Roses<sup>2, 3</sup>, B.L. Scott<sup>1, 3</sup>, K. Fujiwara<sup>1</sup>, M.A. Pericak-Vance<sup>1, 3</sup>, J.M. Vance<sup>1, 3</sup>, Duke CHG/GlaxoSmithKline Parkinson Disease Genetics Collaboration.* 1) Ctr Human Genetics, Box 2903, Duke Univ, Durham, NC; 2) GlaxoSmithKline, Research Triangle Park, NC; 3) Duke CHG/GlaxoSmithKline Parkinson Disease Genetics Collaboration.

Parkinson disease (PD) is the second most common neurodegenerative disorder and is characterized by resting tremor, rigidity, bradykinesia, and gait disturbances. Its etiology remains unknown but the most common, late-onset form of PD is believed to result from the interaction of environmental and genetic factors. We previously detected linkage of late-onset PD with a region on chromosome 17 encompassing the microtubule-associated protein Tau, and found association of the Tau H1 haplotype with risk of idiopathic PD in a family-based study, which has been replicated by other groups in subsequent case-control studies. To subdivide the H1 haplotype in sub-haplotypes potentially even more significantly associated with risk for developing PD, we analyzed 28 single nucleotide polymorphisms (SNPs) in the Tau region. These SNPs are located in or flank the corticotropin-releasing hormone receptor 1, presenilin homolog 2, Tau, Saitohin, and KIAA1267 genes. Five of these SNPs are haplotype tagging SNPs that capture 96% of all the existing haplotype diversity in this region, replacing the previous H1/H2 haplotypes. Single locus and haplotype association analyses revealed that several SNPs and haplotypes are more significantly associated with PD than the Tau H1 haplotype. To define the region of LD around Tau, we analyzed seven additional SNPs flanking the 28 SNPs mentioned above and an LD map in this region was constructed. Analysis of these seven additional flanking SNPs suggests that the significant area of LD around Tau spans approximately over two megabases. These results will also be useful for association studies in other neurodegenerative diseases reported to be associated with the Tau haplotype such as progressive supranuclear palsy, corticobasal degeneration, frontotemporal lobar degeneration syndromes, and primary progressive aphasia.

**Association of functional mutations within the secreted frizzled-related protein 3 gene (FRZB) with susceptibility to hip osteoarthritis in females.** *J. Loughlin<sup>1</sup>, B. Dowling<sup>1</sup>, L. Marcelline<sup>1</sup>, K. Chapman<sup>1</sup>, Z. Mustafa<sup>1</sup>, C. Ciesielski<sup>2</sup>, D. Carson<sup>2</sup>, M. Corr<sup>2</sup>.* 1) Botnar Research Centre, Univ Oxford, Oxford, United Kingdom; 2) Department of Medicine, University of California San Diego.

Primary osteoarthritis (OA, MIM 165720) is a late-onset arthritis that demonstrates an oligogenic and multifactorial mode of transmittance. We have previously linkage mapped a hip OA susceptibility locus to chromosome 2q with a maximum multipoint LOD score of 1.6 in 378 affected sibling pair (ASP) families. This linkage encompasses the gene encoding FRZB (2q32.1), a Wnt antagonist that regulates chondrocyte development. We have investigated FRZB as the potential 2q susceptibility gene. We identified two common single nucleotide polymorphisms (SNPs) within FRZB that each result in the substitution of conserved arginine residues, in exon 4 (Arg200Trp) and exon 6 (Arg324Gly). The exon 6 mutation had an increased frequency in the 220 female probands from our 378 families compared to 399 age-matched female controls ( $P = 0.04$ ). An increased frequency was also observed in an independent cohort of 338 female hip cases ( $P = 0.04$ ). The odds ratio for the mutant allele was 1.5. Haplotype analysis revealed a significant difference between all 558 affected females and the female controls ( $P = 0.002$ ) whilst the possession of a mutant allele of both SNPs was a particular risk factor (odds ratio = 3.6). A partitioning analysis demonstrated that the original linkage was accounted for by those female families in which at least one copy of the two FRZB mutations was segregating. Functional studies demonstrated that the Arg324Gly substitution reduced the ability of the FRZB protein to antagonise Wnt signalling. This antagonism was further reduced in the compound mutant. This data implies that functional mutations within FRZB confer susceptibility for hip OA in females.

**DRB1 gene and Graves' disease in children: case-control and family-based studies.** *Y-J. Lee<sup>1, 2, 4</sup>, C-Y. Huang<sup>1</sup>, M-R. Chen<sup>1</sup>, F-S. Lo<sup>3</sup>, C-H. Lin<sup>1</sup>, S-G. Tsan<sup>2</sup>, C-W. Dang<sup>2</sup>, H-F. Liu<sup>2</sup>, C-C. Chu<sup>2</sup>, M. Lin<sup>2</sup>.* 1) Dept Pediatrics & Medical Res, Mackay Memorial Hosp, Taipei, Taiwan; 2) Dept of Medical Res, Mackay Memorial Hosp, Taipei, Taiwan; 3) Div of Endocrinology, Dept of Medicine, Chang Gung Children's Hospital, Tao-Yuan, Taiwan; 4) Dept of Pediatrics, College of Medicine, Taipei Medical University, Taipei, Taiwan.

We evaluated the association and linkage between the DRB1 gene and Graves' disease in children in both population-based and family-based settings.

#### **Subjects and Methods**

120 unrelated children with Graves' disease (age at diagnosis 9.43.1 years), their parents (66 families), and 190 healthy adults were enrolled. The subjects were Chinese living in Taiwan. The study was carried out after the approval of the local ethnic committee and written informed consents given by the individuals or guardians. Genotyping of the DRB1 gene was by PCR-SBT and direct sequencing. **Statistical analysis** Patients and controls positive for an allele were compared by the  $\chi^2$  test. TDT was used to test the data from families. Corrected p values (pc) were calculated using the Bonferonni inequality method. Statistical significance was defined as pc 0.05.

#### **Results**

Table 1. Alleles frequencies of the DRB1 gene in children with Graves' disease and adult controls (only alleles with pc0.05 listed)

DRB1	Pt(240) %	Ct(380) %	RR	95% CI	pc
09012	79	32.9	59	15.5 2.67	1.8-3.9 5.2E-6

49 parents were heterozygous for DRB1\*09012. Among them, 40 (81.6%) were transmitted and 9 (18.4%) not transmitted with an RR of 4.44 (pc = 8.5E-5).

#### **Conclusion**

This study demonstrates that DRB1\*09012 confers a risk of Graves' disease in children.

**Fine mapping of a candidate susceptibility region on chromosome 11 in Ankylosing Spondylitis.** *J. Lee<sup>1</sup>, J.D. Reveille<sup>2</sup>, L. Luo<sup>1</sup>, J. Bruckel<sup>3</sup>, M. Weisman<sup>4</sup>, M.A. Khan<sup>5</sup>, R. Inman<sup>6</sup>, H.R. Schumacher<sup>7</sup>, W.P. Maksymowych<sup>8</sup>, T.M. Martin<sup>9</sup>, J.T. Rosenbaum<sup>9</sup>, D.T.Y. Yu<sup>4</sup>, M. Stone<sup>6</sup>, L. Jin<sup>1</sup>.* 1) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Division of Rheumatology, University of Texas - Houston, Houston, TX; 3) University of Toronto, Toronto; 4) University of California-Los Angeles, Los Angeles, CA; 5) Case-Western Reserve University, Cincinnati, OH; 6) University of Toronto, Toronto, ON, Canada; 7) University of Pennsylvania, Philadelphia, PA; 8) University of Alberta, Edmonton, AB, Canada; 9) Oregon Health & Science University, Portland, OR.

A genomewide scan that we recently conducted in families with two or more siblings with ankylosing spondylitis (AS) has implicated a broad region on the long arm of chromosome 11 (11q) spanning 68 cM continuously from 88.89 cM to 156.86 cM with the strongest peak at 125.84 cM in susceptibility to AS. The purpose of this study is to carry out fine mapping of this broad region in order to confirm and extend our previous findings and attempt to more precisely localize the susceptibility region(s). In addition to the nine markers on chromosome 11q typed in the ABI PRISM Linkage Map MD-10, an additional nine microsatellite markers flanking three potential susceptibility regions on chromosome 11q identified in the genomewide scan were typed. Two point and multi-point non-parametric linkage analyses were conducted using the NPL-all statistic implemented in GeneHunter 2.1. Overall 269 affected sibling pairs of primarily European ancestry concordant for AS by modified New York criteria with available sacroiliac radiographs from 121 pedigrees were genotyped. The linkage in this region is supported by multi-point analysis where positive linkage is seen at two regions, one spanning 15 cM continuously from 109.3 cM to 124.7 cM with the strongest peak at 118.5 cM ( $p = 0.014$ , NPL=2.191) and a second spanning 10 cM between 135.6 and 145.4 cM with the strongest peak at 141.9 cM ( $p=0.005$ , NPL score = 2.612). These data strongly suggest the presence of at least one (and possibly two) region(s) on chromosome 11q involved in susceptibility to AS.

**Fine mapping of candidate susceptibility regions in Ankylosing Spondylitis on Chromosome 6q25.** *J. Luo<sup>1</sup>, J.D. Reveille<sup>2</sup>, J. Lee<sup>1</sup>, J. Bruckel<sup>3</sup>, M. Weisman<sup>4</sup>, M.A. Khan<sup>5</sup>, R. Inman<sup>6</sup>, H.R. Schumacher<sup>7</sup>, W.P. Maksymowych<sup>8</sup>, T.M. Martin<sup>9</sup>, D.T.Y. Yu<sup>4</sup>, M. Stone<sup>6</sup>, J.T. Rosenbaum<sup>9</sup>, L. Jin<sup>1</sup>.* 1) Center for Genome Information, Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Division of Rheumatology, University of Texas - Houston, Houston, TX; 3) Spondylitis Association of America, Sherman Oaks, CA; 4) University of California-Los Angeles, Los Angeles, CA; 5) Case-Western Reserve, Cleveland, OH; 6) University of Toronto, Toronto, ON; 7) University of Pennsylvania, Philadelphia, PA; 8) University of Alberta, Edmonton, AB, Canada; 9) Oregon Health & Science University, Portland, OR.

A genomewide scan that we have recently conducted in families with two or more siblings with ankylosing spondylitis (AS) has implicated a region on the long arm of chromosome 6 (6q) spanning 24 cM around 154.10 cM in susceptibility to AS in addition to the significant contribution of the MHC located on the short arm of chromosome 6 (6p21.3). The purpose of this study is to carry out fine mapping of this region in order to confirm and extend our previous findings and attempt to more precisely localize the susceptibility region(s). In addition to the three markers in this region on chromosome 6q typed in the ABI PRISM Linkage Map MD-10, an additional five microsatellite markers flanking this potential susceptibility region on chromosome 6q identified in the genomewide scan were typed. Two point and multi-point non-parametric linkage (NPL) analyses were conducted using the NPL-all statistic implemented in GeneHunter v.2. Overall 269 affected sibling pairs of primarily European ancestry concordant for AS by modified New York criteria with available sacroiliac radiographs from 121 pedigrees were genotyped. The linkage in this region is supported by multi-point analysis where positive linkage is seen at a region, one spanning 33.4 cM continuously from 144.5 cM to 177.9 cM with the strongest peak at 154.1 cM ( $p = 0.003$ ,  $NPL=2.617$ ). To conclude, in addition to the MHC, these data strongly suggest the present of another region on chromosome 6, located on the long arm (around 6q25) involved in susceptibility to AS.

**Family-based associations between the angiotensin-converting-enzyme gene insertion/deletion polymorphism and multiple cardiovascular risk factors in Chinese.** X. He, D. Zhu, G. Wang, H. Zhou, W. Zhang. Shanghai Inst of Hypertension, Shanghai 2nd Med. University, Shanghai, China.

**Objectives:** To investigate whether the insertion/deletion (I/D) polymorphism of the angiotensin-converting-enzyme gene (ACE) is associated with multiple cardiovascular risk factors in Chinese hypertensive nuclear families. **Methods:** Genotyping of ACE I/D polymorphism was performed in a total of 1248 Chinese individuals from 304 hypertensive nuclear families. Family-based association tests including transmission disequilibrium tests (TDT and Sib-TDT) and quantitative trait locus (QTL) analysis were applied to interrogate the effects of the ACE I/D polymorphism on both hypertension status and parameters of several other cardiovascular risk factors including obesity, hyperlipidemia and hyperglycemia. **Results:** Weak transmission disequilibrium was detected between hypertension status and ACE I/D polymorphism ( $P=0.048$  for TDT and  $P=0.038$  for Sib-TDT, respectively). Significant association between ACE D allele and body mass index (BMI) was demonstrated in women but not in men, and the analysis favored an additive ( $P=0.007$ ) or a dominant model ( $P=0.002$ ) of inheritance. No association of ACE I/D polymorphism with plasma triglycerides, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and fasting glucose was found. **Conclusion:** Our family-based study suggests that the ACE I/D polymorphism plays a weak role in the development of EH; and the D allele of ACE was significantly associated with a higher BMI in Chinese women but not in men.

**A polymorphism in the promoter region of catalase gene is associated with essential hypertension in Chinese and demonstrates differential transcription activity.** *Y. Li<sup>1,2</sup>, X. Zhang<sup>2</sup>, Z. Wang<sup>1,2</sup>, Y. Li<sup>1</sup>, D. Zhu<sup>3</sup>, W. Huang<sup>2</sup>, L. Jin<sup>1,4</sup>.* 1) School of Life Sciences, Fudan University, Shanghai, China; 2) Chinese National Human Genome Center at Shanghai, Shanghai, China; 3) Shanghai Institute of Hypertension, Shanghai Second Medical University, Shanghai, China; 4) Center for Genome Information, Department of Environmental Health, University of Cincinnati.

Catalase is an important antioxidant enzyme and we showed previously that a polymorphism at its promoter region, -844C/T, is associated with essential hypertension in an isolated Chinese population in Anhui Province. We extended the case-control studies to an outbred population including 1,006 individuals from Shanghai area (average age 549.66 yo, 482 affected/524 controls). The results confirmed the association between genotype frequencies and phenotype ( $P = 0.01$ ), and the association becomes more evident after age stratification ( $P=0.006$  at age between 45-55 yo). We further studied the effect of variation -844C/T on catalase expression. The expression of reporter gene luciferase is analyzed in several cell lines transfected with construct vectors containing 1.2Kb catalase upstream sequence with either C or T variant. It was observed that the expression of luciferase increased by 47.5%, 132% and 56.58% separately transfected with the T variant compared with the C variant in ECV-304, 293, and HeLa cell lines. Furthermore, the results of western blot showed an increase trend of catalase protein in the individuals carrying T/T, T/C to C/C in blood.

**High-density linkage disequilibrium mapping at the 15q12 GABA receptor subunit cluster identifies association in autism.** *J.L. McCauley<sup>1</sup>, L.M. Olson<sup>1</sup>, R. Delahanty<sup>1</sup>, T. Amin<sup>1</sup>, E.L. Organ<sup>1</sup>, E.L. Nurmi<sup>1</sup>, M.M. Jacobs<sup>1</sup>, S.E. Folstein<sup>2</sup>, J.L. Haines<sup>1</sup>, J.S. Sutcliffe<sup>1</sup>.* 1) Program in Human Genetics, Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN; 2) Department of Psychiatry, Tufts University/New England Medical Center.

Autism is a complex genetic neuropsychiatric condition characterized by deficits in social interaction and language and patterns of repetitive or stereotyped behaviors and restricted interests. Chromosome 15q11-q13 is a candidate region for autism susceptibility based on observations of chromosomal duplications of this region in a small percentage of affected individuals and findings of linkage and association. We have performed linkage disequilibrium (LD) mapping across a 1-Mb interval containing a cluster of GABA<sub>A</sub> receptor subunit genes (GABRB3, GABRA5 and GABRG3) thought to be good positional and functional candidates. This region has demonstrated increased evidence for linkage in phenotypic subsets of autism (Shao et al. 2003; Nurmi et al. 2003). Intermarker LD was measured for each of 59 single nucleotide polymorphism (SNP) markers spanning this region, corresponding to an average SNP spacing of ~1 per 17.7 kb. Examination of these data allowed identification of haplotype blocks across the interval, and these blocks were characterized for common (>5%) haplotypes present in our population. Using this information, individual SNPs and multi-SNP haplotypes were examined for evidence of allelic association to autism, using a dataset of 163 combined multiplex and simplex families. Analysis revealed nominally significant (P<0.05) association at 11 of the 59 markers, corresponding to three sites in GABRB3, two in GABRA5 and one in GABRG3. Findings in GABRB3 and GABRA5 are correlated with the position of increased linkage in this region. While ultimate interpretation will require replication in independent samples, these studies suggest that multiple autism risk alleles are present in this region on chromosome 15q11-q13.

**Provisional Mapping of an Adult-Onset Primary Open Angle Glaucoma (POAG) Locus to 5q33-q35. S.**

*MONEMI*<sup>1</sup>, *A. CHILD*<sup>2</sup>, *A. DASILVA*<sup>1</sup>, *O. LEHMANN*<sup>3</sup>, *G. SPAETH*<sup>3</sup>, *R.P. CRICK*<sup>4</sup>, *M. SARFARAZI*<sup>1</sup>. 1) SURGERY, UNIV OF CONNECTICUT, HEALTH CE, FARMINGTON, CT; 2) ST. GEORGES HOSPITAL MEDICAL SCHOOL, LONDON, UK; 3) WILLS EYE HOSPITAL, PHILADELPHIA, PA; 4) INTERNATIONAL GLAUCOMA ASSOCIATION, LONDON, UK.

Primary Open Angle Glaucoma is one of the two major causes of blindness worldwide. In our continuous effort to map and clone new POAG genes, in this study we used two extended adult-onset high-pressure glaucoma families and scanned them for the entire genome. The first family had 108 members in four generations (53 sampled) and the second 99 in five generations (40 sampled). A Genome scan was carried out with ABI/CHLC florescent linkage panels. Genotypic data was entered into DMS and subsequently exported to the CYRILLIC program for haplotype analysis. The most likely inherited haplotypes were constructed manually for each chromosome. Genotyping of over 350 polymorphic DNA markers in family-1 and over 1,000 markers in family-2 excluded linkage to all previously reported POAG loci of GLC1A to GLC1F but identified potential regions on chromosomes 1, 3, 5, 6, 7, 8, 10, 15 and 16. However, additional saturation mapping and haplotype analysis ruled out linkage to all of these regions, except for chromosome 5q. Genotypic data from genome wide scan and saturation mapping showed that all 7-living affected members of family-1 consistently shared a common haplotype for 45 consecutive DNA markers from D5S498 to D5S1466. Initially, family-2 shared a smaller portion of this region but further saturation mapping revealed recombination in two affected individuals thus excluding it from this region. Study of additional 7 adult-onset POAG pedigrees with 5 or more living affected subjects per kindred showed that affected members of only 2 families segregate for the same region on 5q. Significant positive LOD scores were obtained for a number of DNA markers (i.e., Z of 3.22 at theta=0 for D5S436). Linkage study of more POAG families to this region is still needed to confirm this initial observation and to narrow down its critical region. In conclusion, a new locus for adult-onset POAG is provisionally mapped to the 5q33-5q35 region. Supported by EY-09947 and M01RR-06192.

**Genome-wide Scan of Reading Ability in Affected Sibling Pairs with Attention-Deficit/Hyperactivity Disorder: Unique and Shared Genetic Effects.** *S.K. Loo<sup>1,2</sup>, S.E. Fisher<sup>4</sup>, C. Francks<sup>4</sup>, M.N. Ogdie<sup>3</sup>, I.L. MacPhie<sup>4</sup>, M. Yang<sup>1,2</sup>, J.T. McCracken<sup>2</sup>, J.J. McGough<sup>2</sup>, S.F. Nelson<sup>3</sup>, A.P. Monaco<sup>4</sup>, S.L. Smalley<sup>1,2</sup>.* 1) Center for Neurobehavioral Genetics, University of California, Los Angeles, CA; 2) Department of Psychiatry and Biobehavioral Sciences, University of California, Los Angeles; 3) Department of Human Genetics, University of California, Los Angeles; 4) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, U.K.

Attention Deficit/Hyperactivity Disorder (ADHD) and Reading Disability (RD) are common highly heritable disorders of childhood, which frequently co-occur. Data from twin and family studies suggest that this overlap is, in part, due to shared genetic underpinnings. Here, we report the first genome-wide linkage analysis of measures of reading ability in children with ADHD, using a sample of 233 affected sibling pairs who previously participated in a genome-wide scan for susceptibility loci in ADHD. Quantitative trait locus (QTL) analysis of a composite reading factor defined from three highly correlated reading measures, identified suggestive linkage (multipoint maximum lod score,  $MLS > 2.2$ ) in four chromosomal regions. Two regions (16p, 17q) overlap those implicated by our previous genome-wide scan for ADHD in the same sample, one region (2p) provides replication for an RD susceptibility locus, and one region (10q) falls ~35cM from a modestly highlighted region in an independent genome-wide scan of siblings with ADHD. Investigation of an individual reading measure of Reading Recognition supported linkage to putative RD susceptibility regions on chromosome 8p ( $MLS = 2.4$ ) and 15q ( $MLS=1.38$ ). Thus, the data support the existence of genetic factors that have pleiotropic effects on ADHD and reading ability as suggested by shared linkages on 16p, 17q and possibly 10q but also those that appear to be unique to reading as indicated by linkages on 2p, 8p and 15q that coincide with those previously found in studies of RD.

**A common polymorphism in the upstream promoter (P2) of the HNF4 gene on chromosome 20q is associated with type 2 diabetes (T2DM) and appears to account for the evidence for linkage in an Ashkenazi Jewish**

**population.** *L.D. Love-Gregory<sup>1</sup>, J. Wasson<sup>1</sup>, J. Ma<sup>1</sup>, C.H. Jin<sup>2</sup>, B.K. Suarez<sup>2</sup>, M.A. Permutt<sup>1</sup>.* 1) Div of Metabolism; 2) Department of Psychiatry, Washington Univ Sch Medicine, St. Louis, MO.

We reported linkage of T2DM to chromosome 20q in a genome scan of 267 multiplex Ashkenazi Jewish families. To identify a specific locus contributing to the linkage signal, 100 SNPs across a 7.3 Mb region (1 lod) around microsatellite marker D20S107 have been evaluated by an association study. This region encompasses HNF4, a transcription factor that has been shown to play a role in the expression of genes necessary for glucose transport and metabolism. Defects in the HNF4 gene have been shown to cause one type of maturity onset diabetes of the young (MODY). However, mutation analysis in adult T2DM subjects has not identified any association or causative variants. Here, the extent of linkage disequilibrium across 78kb of the HNF4 gene including an alternative upstream promoter (P2), the major regulator of expression in pancreatic  $\beta$ -cells, was determined. Haplotype block structure was assessed by typing 19 SNPs in 35 Ashkenazi trios. Nine haplotype tag SNPs (htSNP) were identified and genotyped in 275 T2DM subjects and 342 non-diabetic controls for case-control analysis. A htSNP 3.9 kb upstream of P2 occurred in 26.9% of cases vs. 20.3% in controls (Odds Ratio 1.45,  $p < 0.008$ ). Variants in absolute linkage disequilibrium with ours were independently found to be associated in a large Finnish T2DM case-control study (Odds Ratio 1.31,  $p < 0.016$ ) (K.L. Mohlke and F. S. Collins, personal communication). Subsequently, linkage to chromosome 20q was re-examined by conditioning on the genotypes of the htSNP near the P2 promoter. A maximum z-score of 2.05 was originally observed at D20S195 and this increased to 3.88 when only those families in which the proband carried at least one risk allele ( $n=91$ ) were considered. Alternatively, a z-score of 0.33 was observed in those families in which the probands did not carry the risk allele ( $n=115$ ). The results of these studies now suggest that this polymorphism, or a variant in linkage disequilibrium with it, may alter expression of HNF4 and predispose to T2DM.

**Follow-Up to a Second Generation Genomic Screen for Multiple Sclerosis.** *S.J. Kenealy<sup>1</sup>, Y. Bradford<sup>1</sup>, N. Schnetz-Boutaud<sup>1</sup>, J.R. Oksenberg<sup>2</sup>, S.L. Hauser<sup>2</sup>, L.F. Barcellos<sup>2</sup>, R.R. Lincoln<sup>2</sup>, S. Schmidt<sup>3</sup>, M.A. Pericak-Vance<sup>3</sup>, J.L. Haines<sup>1</sup>.* 1) Vanderbilt University, Nashville, TN; 2) University of California, San Francisco, CA; 3) Duke University, Durham, NC.

Multiple sclerosis (MS) is a debilitating neuroimmunological disease that affects an estimated 400,000 individuals in the United States. Genomic screens have identified numerous regions of interest for MS loci. However, with the exception of the human leukocyte antigen (HLA) locus, studies have failed to identify genes that modulate MS disease expression.

In conjunction with the French Multiple Sclerosis Genetics Group, we recently completed a second generation genomic screen for MS with 361 microsatellite markers in 245 multiplex families consisting of 344 affected sibpairs and 112 other affected relative pairs. In addition to the HLA-DR locus, the strongest signals from our screen were generated at 1q42, 2q32, 9q34, 13q12, 18p11, and 19q13 (lod scores >2.0 in MLOD or multipoint analyses).

A significant expansion of our MS dataset (an additional 36 families consisting of 50 affected relative pairs) was used for follow-up studies in these six non-HLA regions. Regions 1q42 and 9q34 were prioritized for follow-up based on initial strong results from our screen and congruency with other recent screens for MS and other autoimmune disorders. SNPs located at ~1 cM intervals extending 10 cM to each side of markers exhibiting peak linkage signals from our screen were genotyped in the expanded dataset.

Five SNPs in the 1q42 region generated MLOD scores 1.0, with the highest score being an MLOD=2.52. One SNP in the 1q42 region generated significant P values for allelic association using the PDT (P=0.01) and Transmit (P=0.05). Four SNPs in the 9q34 region generated MLODs 1.0. One SNP in the 9q34 region generated a significant P value using the PDT (P=0.01). These analyses continue to support the localization of MS genes in 1q42 and 9q34. Analysis of additional SNPs is ongoing to narrow intervals of interest in all six follow-up regions.

**Interindividual variation of efficacy of sublingual nitroglycerin is associated with a polymorphism of ALDH2 in Chinese.** *L. Jin<sup>1,2,3</sup>, Y. Li<sup>2</sup>, W. Jin<sup>4</sup>, H. Sheng<sup>3</sup>, Y. Liu<sup>4</sup>, G. Lu<sup>4</sup>, J. Yu<sup>4</sup>, D. Lu<sup>1</sup>, D. Harrison<sup>1</sup>, W. Huang<sup>3</sup>.* 1) Center for Genome Information, Department Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) School of Life Sciences, Fudan University, Shanghai, China; 3) Chinese National Human Genome Center at Shanghai, Shanghai, China; 4) Ruijin Hospital, Shanghai Second Medical University, Shanghai, China.

Nitroglycerin has been the first choice in the treatment and/or management of acute attacks of angina pectoris among antianginal drugs. The mechanism of its vasodilator effect was not fully understood until it was recently showed that its biotransformation is catalyzed by aldehyde dehydrogenase (ALDH2). A well-characterized G-to-A polymorphism at 12th exon of ALDH2, which virtually eliminates the activity of the enzyme in its carriers, has the highest prevalence in Asian populations. We report here that interindividual variation of the efficacy of sublingual nitroglycerin is significantly associated with this ALDH2 polymorphism in Chinese ( $c = 7.59$ ,  $P < 0.01$ ,  $n = 80$ ). We hypothesize that the presence of the A allele (ALDH2\*2) is partially responsible to the failure or reduced response to nitroglycerin; therefore genetic factor should be involved in the consideration of the administration of nitroglycerin especially in oriental populations.

**Association study of polymorphisms in two metabotropic glutamate receptor genes, *GRM3* and *GRM8* with schizophrenia.** H. Shibata<sup>1</sup>, Y. Fujii<sup>1</sup>, M. Takaji<sup>1</sup>, H. Takaki<sup>1</sup>, R. Kikuta<sup>1</sup>, C. Makino<sup>1</sup>, A. Tani<sup>1</sup>, N. Hirata<sup>1</sup>, A. Shibata<sup>1</sup>, H. Ninomiya<sup>2</sup>, N. Tashiro<sup>3</sup>, Y. Fukumaki<sup>1</sup>. 1) Research Center for Genetic Information, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; 2) Fukuoka Prefectural Dazaifu Hospital Psychiatric Center, Fukuoka, Japan; 3) Department of Neuropsychiatry, Grad Sch of Med Sci, Kyushu University, Fukuoka, Japan.

Based on the glutamatergic hypothesis for the pathogenesis of schizophrenia, we have been conducting a systematic study of associations of glutamate receptor (GluR) genes with the disease. Here we report association studies on two metabotropic GluR genes, *GRM3* and *GRM8*. ***GRM3***: We examined 6 iSNPs evenly distributed in the entire *GRM3* region (270 kb) (SNP1-SNP6). We observed a significant association of SNP4 with schizophrenia (allele:  $P = 0.011$ ; genotype:  $P = 0.038$ ), although neither remained significant after Bonferroni correction. We observed highly significant haplotype associations with the disease in multiple combinations including SNP4 ( $P = 8.3 \times 10^{-4}$ ,  $P_{\text{corr}} = 5.0 \times 10^{-3}$  with Bonferroni correction at the lowest). Then we genotyped 10 additional SNPs around SNP4 to examine the LD status of the subregion. We observed significant LD within the 40-kb region containing SNP4. Five out of the 10 SNPs replicated the significant association with schizophrenia ( $P = 0.011$ , at the lowest). We conclude that the susceptibility locus for schizophrenia is located within the 40-kb region in the vicinity of SNP4 in *GRM3*. ***GRM8***: We examined 22 iSNPs evenly distributed in the entire *GRM8* region (800 kb) (SNP1-SNP22). We observed significant associations of schizophrenia with SNP18 (allele:  $P = 0.028$ ; genotype:  $P = 0.012$ ) and with SNP19 (allele:  $P = 0.030$ ; genotype:  $P = 0.013$ ), although neither remained significant after Bonferroni correction. We also observed significant haplotype associations with the disease in the following three combinations: SNP5-SNP6 ( $P = 0.0004$ ,  $P_{\text{corr}} = 0.092$  with Bonferroni correction), SNP4-SNP5-SNP6 ( $P = 0.0075$ ,  $P_{\text{corr}} = 0.015$  with Bonferroni correction) and SNP5-SNP6-SNP7 ( $P = 0.0011$ ,  $P_{\text{corr}} = 0.0022$  with Bonferroni correction). We conclude that susceptibility loci for schizophrenia are situated within or very close to *GRM8*.

**High Density SNP Based Association Study of 21 Candidate Genes in Autism Spectrum Disorder.** *J.L. Stone<sup>1</sup>, S. Tsai<sup>2</sup>, B. Merriman<sup>1</sup>, R.M. Cantor-Chiu<sup>1</sup>, D.H. Geschwind<sup>2</sup>, S.F. Nelson<sup>1</sup>, AGRE Consortium<sup>3</sup>*. 1) Dept. Human Genetics, Gonda, Univ California, Los Angeles, Los Angeles, CA; 2) Dept. Neurology, Program in Neurogenetics and the Neuropsychiatric Institute, Center for Neurobehavioral Genetics; UCLA School of Medicine; 3) Autism Genetic Resource Exchange, Cure Autism Now, Los Angeles, CA.

Autism is a complex disorder identified by impaired language and social skills, restricted repetitive behaviors, and an early childhood onset. Twin and family studies implicate a significant role for genetic etiologies and current evidence suggests that multiple interacting genes are likely causal. We have performed at 10 cM density a whole genome scan in 330 multiplex families from the AGRE consortium (Geschwind et al. 2001). Regions on chromosomes 7q and 17q have presented themselves as likely locations for susceptibility loci, and these regions have been refined by fine mapping with microsatellites. Using these findings as a guide, we chose to test for association to single nucleotide polymorphisms in 21 positional candidate genes. Genes located on chromosome 17 include FLOT2, SLC6A4, CENTA2, NF1, OMG, CDK5R1 and DARPP32. Genes located on chromosome 7 include HIPK2, BRAF, CLECSF5, TBXAS1, CNTNAP2, and ZNF212. We also tested for association in the 5 known or suspected neuroligin genes, since they have been recently implicated in a European sample (Jamain et al. 2003). We performed TDT analysis on 218 parent-child trios obtained through the AGRE Consortium and the NIMH. There are on average 16 SNPs per gene, making an average inter-SNP distance of 5kb with a gene.

**Identification of potential therapeutic targets in complex diseases: evidence for protection from type1 diabetes (T1D) by variants of *CCR2* and *CCR5*.** D. Smyth<sup>1</sup>, J. Howson<sup>1</sup>, R. Twells<sup>1</sup>, P. Badhwar<sup>2</sup>, N. Walker<sup>1</sup>, H. Rance<sup>1</sup>, B.J. Barratt<sup>1</sup>, D. Savage<sup>3</sup>, D. Carson<sup>3</sup>, E. Tuomilehto-Wolf<sup>4</sup>, J. Tuomilehto<sup>4</sup>, C. Guja<sup>5</sup>, C. Ionescu-Tirgoviste<sup>5</sup>, D. Undlien<sup>6</sup>, K. Rønningen<sup>6</sup>, G. Stewart<sup>2</sup>, J.A. Todd<sup>1</sup>. 1) JDRF/WT Diabetes and Inflammation Laboratory, CIMR, University of Cambridge, UK; 2) Westmead Hospital, Sydney; 3) Belfast City Hospital, Northern Ireland; 4) National Public Health Institute, Finland; 5) Clinic of Diabetes, Romania; 6) National Institute of Public Health, Norway.

The association analysis of functional candidate genes still dominates the dissection of complex traits. Classically, the common (major) allele of a variant is considered as the wild type with normal function, and the rarer (minor) allele as the susceptibility allele. However, the minor allele of some variants may, conversely, provide resistance to disease. If the effect of these rare protective variants can be mimicked by therapeutic agents it may be of benefit to a large proportion of the population. Rare functional variants in the closely linked chemokine receptor genes, *CCR5* and *CCR2*, have already been shown to provide resistance to HIV and delay progression to AIDS, respectively. As these genes are involved in chemoattraction in the immune system we consider them to be candidates for immune-mediated diseases such as T1D and, therefore, genotyped the variants *CCR5*-32 and *CCR2*-V64I in approximately 3,700 T1D families. Our results suggest that the minor alleles of these polymorphisms, which are not in strong LD, are associated with lower T1D risk (RR for *CCR5*-32/32 genotype =0.55 [95%CI 0.4-0.8],  $P=0.005$  and OR for I allele of *CCR2*-V64I =0.85 [95%CI 0.7-0.9],  $P=0.006$ ). Stepwise conditional logistic regression indicated that both variants are required to explain the association of the region. The blocking of the interactions of chemokines and their receptors is a major target in the development of drugs against HIV and AIDS. If the association of *CCR5* and *CCR2* with T1D is confirmed in a large, independent data set, anti-*CCR5*/*CCR2* drugs could also be considered in the on-going development of therapeutics to prevent rejection of pancreatic cells in diabetic transplant recipients.

**Serotonin and dopamine system candidate gene studies of novelty seeking, extroversion and neuroticism in a large unselected population-based sample.** *J. Vittum, C.A. Prescott, J. Kuhn, P.F. Sullivan, K.S. Kendler, B. Riley.*  
Dept. of Psychiatry, Virginia Commonwealth University, Richmond, VA.

Serotonin and dopamine systems have been investigated in relation to personality traits with inconsistent results. We attempted to detect association and interaction between 5HT2A T102C, 5HT2C Cys23Ser, HTT promoter long/short and DRD2 Taq1A candidate gene polymorphisms and 3 personality traits. Our sample included 572 unrelated Caucasian participants (male(M)=284, female(F)=288) who were randomly selected from the Virginia Adult Twin Study of Psychiatric and Substance Use Disorders. These individuals had completed a structured psychiatric interview, the 12-item Neuroticism(N) and 8-item Extroversion(E) short forms of the Eysenck Personality Questionnaire and the 18-item Novelty seeking(NS) subscale of the Tridimensional Personality Questionnaire. Participants were unselected for either trait score or clinical criteria. M and F mean scores for NS (m=6.9, f=7.3), E (m=5.0, f=5.2) and N (m=3.4, f=3.1) did not differ significantly. For NS, which is normally distributed, we used general linear modeling (GLM) analysis to test for associations and interactions. We observe significant main effects of DRD2 in M (genotype 1/1 associated with lower NS,  $p=0.048$ ). Models including main effects of DRD2 and 5HT2A in M ( $p=0.026$ ) and DRD2, 5HT2A and HTT in F ( $p=0.017$ ) accounted for more of the variation in NS. In the best fitting model for F, we also detect significant interactions between DRD2 (1/1) and HTT (L/S) increasing NS ( $p=0.035$ ), and HTT (S/S) and 5HT2A (T/T) lowering NS ( $p=0.046$ ). We used logistic regression with recoded categorical trait scores (low vs. all others for E, high vs. all others for N) for skewed and truncated distributions of E and N. For E, we detect a significant main effect of 5HT2A in both M (C/T associated with lower E,  $p=0.011$ ) and F (C/C associated with lower E,  $p=0.028$ ). We also detect a significant main effect of DRD2 in F (2/2 associated with low E,  $p=0.012$ ). For N, we detect a significant main effect of 5HT2C in F only (C/S associated with higher N,  $p=0.012$ ). These results are noteworthy because of our large unselected population-based sample.

**The Role of Gene-Risk Factor Interactions in Predicting Susceptibility to Sporadic Breast Cancer.** *M.D. Ritchie<sup>1</sup>, J.J. Hu<sup>2</sup>, T.R. Smith<sup>2</sup>, J.H. Moore<sup>1</sup>*. 1) Program in Human Genetics, Vanderbilt University, Nashville, TN; 2) Department of Cancer Biology, Wake Forest University, Winston-Salem, NC.

Variability in DNA repair genes may contribute to human cancer risk. An important question is whether breast cancer risk can be predicted by knowledge about variation in DNA repair genes and/or hypothesized breast cancer risk factors. Previous studies suggest that DNA repair genes have minimal main or independent effects. The goal of this study was to determine whether interactions among variations in DNA repair genes and/or risk factors are associated with sporadic breast cancer. We employed the multifactor data reduction (MDR) method to detect interactions in a sample of 162 breast cancer cases and 108 controls without a family history of breast cancer. MDR is a powerful new statistical approach used to detect nonlinear gene-gene interactions. MDR evaluates all possible combinations of genotypes and risk factors for their ability to predict disease status using cross-validation and permutation testing. We measured seven polymorphisms in six DNA repair genes: XRCC\_1, XRCC\_3, APE\_5, XPD\_23, GSTM1, and GSTT1. We also measured three risk factors: age at first live birth, age of menarche, and parity. We detected a statistically significant interaction between the XRCC\_3, APE\_5, and XPD\_23 genes with a prediction error of 39% ( $p=0.027$ ) in addition to an interaction between age of first live birth and parity with a prediction error of 31% ( $p<0.001$ ). Interestingly, the simultaneous analysis of all genes and risk factors identified a single best model with a prediction error of 30% ( $p=0.022$ ) that consisted of the same three genes and two risk factors identified in the separate analyses. These results suggest that combined information about genes and traditional risk factors provides more information for predicting risk of breast cancer than either alone. This study illustrates the importance of considering high-order gene-gene and gene-risk factor interactions in studies of complex diseases such as sporadic breast cancer when the independent main effects of each gene and risk factor are relatively small.

**Fine mapping of 17q25 supports linkage with Gilles de la Tourette Syndrome.** *P. Paschou<sup>1</sup>, A.J. Pakstis<sup>1</sup>, W.C. Speed<sup>1</sup>, M.M.C. DeMille<sup>1</sup>, J.R. Kidd<sup>1</sup>, V. Ruggieri<sup>1</sup>, R. Kurlan<sup>2</sup>, D.L. Pauls<sup>3</sup>, K.K. Kidd<sup>1</sup>.* 1) Department of Genetics, Yale U. School of Medicine, New Haven, CT; 2) Department of Neurology, U. Rochester Medical Center, Rochester, NY; 3) Department of Psychiatry, Massachusetts General Hospital, Boston, MA.

The mode of inheritance of Gilles de la Tourette Syndrome (GTS) is currently thought to involve multiple genes interacting with environmental factors. 17q25 is one of the regions for which indications for linkage with the disorder have been provided by a whole genome scan and unpublished reports by various independent investigators associated with the Tourette Syndrome Association International Consortium for Genetics. We report here results of a high resolution study of 17q25 in an effort to replicate the initial findings and reduce the large candidate susceptibility interval. We studied four large families from Canada, Kansas, Michigan, and Oregon (344 individuals, 120 affected members) using 22 microsatellite markers at an average density of 4 cM. In order to enhance the map density, 12 SNPs were also typed. The region covered at high density extends from D17S933 to 17qter. For the Oregon family and the largest branch of the Canadian family seven additional microsatellite markers spanning the rest of chromosome 17 were also studied. Analyses included TDT and non-parametric linkage analysis (GENEHUNTER). Multipoint analysis for the Oregon family gave a Zall score of 2.2 at D17S928 ( $p=.003$ ). The largest branch of the Canadian family also gave results suggestive for linkage in this region (rs733342, Zall=2,  $p=.02$ ). A smaller broad peak near D17S933, 65 cM upstream, was also found. TDT results in this pedigree indicated an association at D17S784. In analyses combining the Oregon and the largest branch of the Canadian kindred the peak score was even higher and close to rs733342 ( $p=.00007$ ). These results reinforce the evidence of the whole genome scan that distal 17q is one of the regions of interest for GTS. Additional markers and haplotype analysis will help further restrict the candidate region. (Supported in part by NIH NINDS NS40024; P.Paschou is supported by a Tourette Syndrome Association grant.).

**Association of human SCN6A gene with essential hypertension in Chinese.** *K. Zhang<sup>1</sup>, D. Zhu<sup>1</sup>, X. He<sup>1</sup>, G. Wang<sup>1</sup>, W. Huang<sup>2</sup>.* 1) Shanghai Inst Hypertension, Shanghai 2nd Med University, Shanghai, China; 2) Chinese National Human Genome Center, Shanghai, China.

**Objectives** To identify the single nucleotide polymorphisms (SNPs) in the regulatory and coding regions of human SCN6A sodium channel, voltage-gated, type VI, alpha polypeptide gene and to detect the possible relationship between some of these SNPs and essential hypertension (EH) in Chinese. **Methods** The promoter region, exons, as well as part of the introns of SCN6A gene were sequenced by a fluorescent labeling automatic sequencing method to identify and characterize the SNPs in Chinese population. SNP genotyping was performed by PCR-RFLP or direct DNA sequencing in unrelated EH patients and normotensive controls from a Chinese Han population residing in Shanghai area. Case-control studies on seven unlinked SNPs were first carried out in 96 patients and 96 normotensive controls. The positive finding was further verified in an extended study containing 288 patients and 288 controls. **Results** Thirty-two SNPs were identified through a 13132 bp sequencing of SCN6A gene. Among them, seven were in regulatory region, ten in coding regions, one in 3'UTR and fourteen in introns. Thirty SNPs were novel SNPs, and a cSNP in exon 18 (SNP021) was associated with hypertension. **Conclusion** The SNP021 in the gene SCN6A is associated with essential hypertension of Chinese Han population in Shanghai and the role of SCN6A gene in hypertension deserved to be further analyzed.

**Genetic variations in kynureninase gene are associated with essential hypertension in Chinese.** *D. Zhu<sup>1</sup>, X. He<sup>1</sup>, K. Zhang<sup>1</sup>, Y. Zhang<sup>1</sup>, G. Wang<sup>1</sup>, W. Huang<sup>2</sup>.* 1) Shanghai Inst Hypertension, Shanghai 2nd Med. University, Shanghai, China; 2) Chinese National Human Genome Center at Shanghai, Shanghai, China.

Essential hypertension (EH) is a common cardiovascular disorder and occurs as a consequence of a complex interplay of multiple genetic alterations and environmental factors. Our previous genome-wide scan study has mapped a candidate locus for EH on chromosome 2q14-q23. One of the functional candidate genes located within this region is the kynureninase gene (KYNU). In order to investigate whether the nucleotide variations in KYNU are associated with hypertension in Chinese, we first sequenced all 14 coding exons and flanking introns, as well as the promotor region of KYNU. Altogether, fourteen single nucleotide polymorphisms (SNPs) were identified, which included 8 novel SNPs. Further studies were focused on two nonsynonymous SNPs (Arg188Gln and Lys412Glu). For a rare Arg188Gln polymorphism (2.29% for allele 188Gln), a genotype-discordant sib-pair study was performed. In the 39 sibling-pairs discordant for the Arg188Gln polymorphism, the siblings carrying one or two 188Gln allele had significant higher systolic blood pressure (SBP) and diastolic blood pressure (DBP) than the siblings homozygous for the 188Arg allele ( $P < 0.001$  for systolic blood pressure and  $P = 0.001$  for diastolic blood pressure, respectively). For a common Lys412Glu polymorphism, a case-control association study was carried out in a total of 672 unrelated hypertensive patients and 477 unrelated normotensive controls. A borderline association between the Lys412Gly genotypes and hypertensive status was detected ( $P = 0.048$ ). This association was observed in the female group ( $P = 0.042$ ), but was not noted in the male group. We therefore hypothesize that genetic variations within KYNU may account for individuals developing EH in Chinese. Our results suggest that the role of kynureninase pathway on blood pressure modulation needs to be further studied.

**A Two Stage Genome-Wide Linkage Scan for Nonsyndromic Cleft Lip with or without Cleft Palate in 220 Filipino Families Shows Evidence of Linkage to 2p21, 6q23, and 8p21.** *R. Schultz<sup>1</sup>, M. Cooper<sup>2</sup>, B. Riley<sup>1</sup>, T. Goldstein<sup>2</sup>, S. Daack-Hirsch<sup>1</sup>, B. Nepomucena<sup>3</sup>, M. Marazita<sup>2</sup>, J. Murray<sup>1</sup>.* 1) Dept Pediatrics Univ Iowa; 2) School Dental Med Univ Pittsburgh; 3) HOPE Found. Philippines.

Non syndromic CLP is a complex disorder resulting from multiple genetic and environmental factors. NS CLP has a birth prevalence of 1 per 500 in the Philippines where large families provide opportunity for gene localization. A two stage genome-wide linkage scan was done on 220 Filipino families with 2 affecteds. Genotyping on 390 microsatellite markers at 10 cM intervals was done on 109 families with 282 affected and 543 unaffected. A second set of 111 families with 285 affected and 566 unaffected was genotyped to replicate the results from the first group. Two- and multi-point parametric linkage analyses (FASTLINK, SIMWALK2) and nonparametric multipoint and TDT analyses (SIMWALK2, FBAT) were performed. The first scan identified 10 regions with LOD 1.0 or  $p < 0.05$  in one or more test. These regions were also positive in the second scan; the pooled results from all 220 families had the most significance for 2p, 6q and 8p. The 8p region has not been previously identified nor does it contain any of the candidate genes widely studied in CLP. Fine mapping of 8p21 has begun in the first family set using SNP TaqMan markers from eight genes (FZD3, SLC8A1, PPP3CC, EPHX2, BNIP3L, EGR3, PPP2R2A and NAT1) within the 20 cM of 8p with LOD1.0. A SNP in PPP3CC with a two-point recessive LOD of 1.91 in the first 109 families is being tested for transmission distortion in the second family set and an additional 500 parent-case triads. Fine mapping of 2p21 and 6q23 is also underway. A meta-analysis of this genome scan and several other CLP genome scans also had positive results for chromosomes 2 and 6 (ASHG presentation by Marazita et al). In summary, we report the largest NS CLP genome scan to date and identify 3 highly positive regions. PPP3CC, a strong candidate gene on 8p, is under intensive scrutiny by direct exon sequencing. For the first time, fine mapping and gene identification appear feasible for NS CLP. This research was supported by NIH grant DE-08559 and the Center for Inherited Disease Research.

**Genetic variation in the Collagen 6A1 and 6A2 genes in individuals with Down Syndrome with and without atrioventricular septal defects.** *R.E. Pyatt<sup>1</sup>, M. Gupte<sup>1</sup>, C. Torfs<sup>2</sup>, G. Capone<sup>3</sup>, K. Dooley<sup>4</sup>, S.B. Freeman<sup>1</sup>, S.L. Sherman<sup>1</sup>.* 1) Dept of Human Genetics, Emory University, Atlanta, GA; 2) Public Health Institute, Birth Defects Study, Emeryville CA; 3) Dept of Pediatrics, Johns Hopkins Medical Institutions, Baltimore, MD; 4) Sibley Heart Center, Dept of Pediatrics, Emory University, Atlanta, GA.

Approximately 40% of individuals with Down syndrome (DS) are born with a congenital heart defect (CHD). Atrioventricular septal defects (AVSD) are the most common of these accounting for 45% of CHD associated with DS while occurring in only 3-4/10,000 live births in the general population. Collagen VI, a heterotrimer of Collagen 6A1 (COL6A1), Collagen 6A2 (COL6A2), and Collagen 6A3 chains, is expressed in vascular smooth muscle, heart, and the connective tissues. The genes for COL6A1 and COL6A2 are located as a cluster on 21q22.3 within a region of chromosome 21 implicated in the causation of DS-associated CHD. Consequently, alteration in the function of these genes or their expression could contribute to the failure of septation in the heart. We undertook a study to identify the genetic variation in the COL6A1 and COL6A2 genes associated with the development of AVSD in DS individuals using a case (DS with AVSD)/control (DS without CHD) approach. Since Collagen VI is present in human and mouse fetal heart, we initiated our studies with the examination of an STR in the COL6A2 gene. Preliminary findings indicated no difference in the allele distribution in our Caucasian population, but a significant difference in an African-American one ( $p=0.02$ ). To determine if this difference is biologically relevant, we identified 5 haplotype-tag SNPs within COL6A1 and 17 in COL6A2 using the data from Patil et al (Patil et al, 2001, Science 294:1719-1722). These SNPs are being analyzed in 36 DS individuals with verified AVSD, 63 DS individuals without CHD, and all respective parents. We are currently finalizing the analysis and data will be examined using both a case/control genotyping comparison as well as a TDT approach modified to account for the non-independence of alleles contributed by the parent in whom the nondisjunction error occurred.

**Linkage and Association Analysis of Neuregulin-1 Gene Variants with Schizophrenia in the Irish Study of High Density Schizophrenia Families.** *D.L. Thiselton<sup>1</sup>, B.T. Webb<sup>1</sup>, R. Ribble<sup>1</sup>, F.A. O'Neill<sup>2</sup>, D. Walsh<sup>3</sup>, K.S. Kendler<sup>1</sup>, B. Riley<sup>1</sup>.* 1) Dept Psychiatry, Virginia Commonwealth Univ, Richmond, VA; 2) Dept Psychiatry, Queens University, Belfast, N. Ireland; 3) Health Research Board, Dublin, Ireland.

The neuregulin 1 gene (*NRG1*) at chromosome 8p21-22 has been implicated as a schizophrenia susceptibility gene in Icelandic and Scottish populations. A number of genome scans and meta-analyses have reported evidence for linkage of schizophrenia to 8p21-22. Analysis of our Irish high density schizophrenia families generated a linkage signal within this interval. Although ~20cM distal to *NRG1*, the shared ancestry between the Icelandic, Scottish and Irish populations led us to investigate *NRG1* variants and appropriate marker haplotypes for over-representation in our Irish familial cases. Singlepoint linkage analysis using 7 *NRG1* markers including the core Icelandic risk haplotype gave no significant results across 4 diagnostic categories. Multipoint analysis incorporating flanking markers D8S137 and D8S283 produced suggestive evidence for linkage peaking at SNP243177 (NPL Z-score=1.75, p=0.04). However, re-analysis incorporating the *NRG1* genotypes into our existing genome scan data showed the linkage signal from *NRG1* to be part of the original peak centred around LPL-D8S258. Analysis of linkage disequilibrium (LD) across the 251kb 7-marker interval revealed 2 separate 'blocks' of LD within our sample, comprising SNPs 255133, 249130 and 243177 (telomeric) and microsatellites 478B14-428, 420M9-1395, D8S1810 and 420M9-116I12 (centromeric, close to Hs.97362). We therefore sought association with disease by considering haplotypes for the 3 SNPs, then the 4 microsatellites within each block. Single marker analysis gave no evidence for association with schizophrenia. Haplotype analysis for the 3 SNPs in LD block 1 and separately, the 4 microsatellites in LD block 2 (in overlapping 2-marker windows), showed no evidence for overtransmission of specific haplotypes to affected individuals. We thus conclude that if *NRG1* does contain susceptibility alleles for schizophrenia, they play a relatively minor role in our Irish family sample.

**Synergistic effect of multiple risk alleles in association study of ADHD.** *L. Park<sup>1</sup>, K. Nummy<sup>2</sup>, M. Huebner<sup>6</sup>, I. Waldman<sup>5</sup>, M. Rappley<sup>4</sup>, J. Nigg<sup>3</sup>, K. Friderici<sup>1, 2</sup>.* 1) Genetics Program, Michigan State University, East Lansing, MI; 2) Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI; 3) Department of Psychology, Michigan State University, East Lansing, MI; 4) Peds/Hum. Dev., Michigan State University, East Lansing, MI; 5) Department of Psychology, Emory University, Atlanta, GA; 6) Department of Statistics and Probability, Michigan State University, East Lansing, MI.

Attention Deficit Hyperactivity Disorder (ADHD) is a behavior disorder with strong heritability (0.7) and a very high prevalence in children (3-5%). Despite the high heritability, genetic studies reported to date have not shown consistent association with single genetic polymorphisms and two genome-wide scans found inconsistent effects with no clear major gene effect. Lack of a major gene effect reinforces the polygenic nature of ADHD and implies minor contributions from a large number of genes. In this study, we examined 8 polymorphisms on 3 genes using family samples of childhood ADHD (62 control, 115 affected). Diagnostic criteria for ADHD were directly evaluated using structured diagnostic interviews and multi-informant standardized rating scales to assess DSM-IV criteria, severity, onset, duration, and impairment. We found significant associations on the DRD4 gene and the SLC6A3 gene. Most interesting was that we noted associations were strengthened by looking at the combined effect of multiple alleles on three genes (DRD4, SLC6A3 and ADRA2A). Most of the polymorphisms did not show any significance using TDT except one SNP on ADRA2A. Our sample population is not skewed according to ethnicity and shows no admixture. It should be also noted that our parent group of affected children shows significantly biased frequencies of risk alleles. It seems that the suitability of using TDT for detecting the effective polymorphisms highly contributing to this very heritable trait may need further clarification depending on the population. Our result suggests that the synergistic effect among genes must be considered in association studies of complex traits.

**Possible association of a 14 kb haplotype block from the receptor activator of NF-kappa B (RANK) gene with susceptibility to rheumatoid arthritis.** *E. Remmers*<sup>1</sup>, *J. Balow, Jr.*<sup>1</sup>, *W. Li*<sup>2</sup>, *I. Aksentijevich*<sup>1</sup>, *A. Lee*<sup>2</sup>, *A. Damle*<sup>2</sup>, *W. Chen*<sup>3</sup>, *C. Amos*<sup>3</sup>, *P. Gregersen*<sup>2</sup>, *D. Kastner*<sup>1</sup>. 1) Genetics and Genomics Branch, NIAMS, Bethesda, MD; 2) North Shore Long Island Jewish Research Institute, Manhasset, NY; 3) Univ of Texas/MD Anderson Medical Center, Houston, TX.

The North American Rheumatoid Arthritis Consortium has collected 512 multiplex rheumatoid arthritis (RA) families containing 581 independent affected sibling pairs and has completed two genome-wide screens, each with half the sib-pairs. A 15 cM region on chr 18 exhibited suggestive evidence for an RA susceptibility locus in both screens (Jawaheer, et al., *Arthritis Rheum* 48:906-916, 2003). A candidate gene within this susceptibility region is the receptor activator of NF-kappa B (RANK, gene symbol: *TNFRSF11A*), a gene involved in osteoclast differentiation and in regulation of apoptosis of immune cells. To evaluate whether variants within this gene might be associated with RA susceptibility, we undertook a case-control haplotype association study using 414 of the RA probands and 373 ethnically matched controls. We genotyped 15 SNPs in this gene. Two haplotypes were inferred for each individual using an expectation maximization algorithm. Linkage disequilibrium (LD) was then measured between each pair of SNPs. The results demonstrated that the RANK gene was organized into four LD blocks. Within each block there were 3-5 common haplotypes. We evaluated whether any haplotype was more prevalent in the RA probands than in the controls. One haplotype of the second LD block of the RANK gene was found at a frequency of 42% in the RA probands and only 36% in the controls (uncorrected p value = 0.01). The outermost limits of this haplotype block included 14 kb of the RANK gene, encompassing a large portion of the first intron and the second exon. Suggestive evidence for an association of the genotypes of a pair of SNPs located within the block was also found. A pedigree disequilibrium test with genotypes from available family members was also suggestive of an RA susceptibility locus in this region. Further studies will be required to confirm these findings and to identify functional variants that confer susceptibility to RA.

**HAPLOTYPE MAPPING OF A COMPLEX DISEASE: SCHIZOPHRENIA AND CHROMOSOME 5Q. P.**

*Sklar<sup>1, 2</sup>, T. Petryshen<sup>1, 2</sup>, A. Kirby<sup>2</sup>, A. Tahl<sup>2</sup>, K. Aldinger<sup>2</sup>, S. Waggoner<sup>2</sup>, A. Verner<sup>3</sup>, T. Hudson<sup>3</sup>, H. Medeiros<sup>4</sup>, E. Lander<sup>2</sup>, M.T. Pato<sup>4</sup>, M.J. Daly<sup>2</sup>, C.N. Pato<sup>4</sup>.* 1) Harvard Medical School, Boston, MA; 2) MIT/Whitehead Institute for Biomedical Research Cambridge, MA; 3) Montreal Genome Center, Montreal, CA; 4) State University of New York, Syracuse, NY.

We describe the results of an aggressive, haplotype-map based screen to positionally identify a schizophrenia gene on chromosome 5q. Focusing on families of Portuguese descent collected in the Azores, Madeira and the Portuguese mainland, we identified maximum evidence of linkage (NPL = 3.1) at marker D5S820. Simulations suggested that this result was at the threshold required to declare significant genome-wide linkage, and when a handful of additional markers were added, the evidence increased to an NPL = 3.55. Genotyping a second smaller set of families confirmed linkage to this region. In addition, the same region of 5q was recently identified in a meta-analysis of published genome scans (importantly not including our own study) as containing a highly significant risk factor of global importance. However, as in most complex diseases, the region implicated by linkage is quite large (30 Mb) and two strategies for follow-up were pursued. We selected a set of 15 high likelihood positional and functional candidate genes for initial linkage disequilibrium mapping. SNPs were selected at a density of ~1/5kb and genotyped in 98 affected-parent trios and 150 cases and ethnically matched controls. Haplotype maps were constructed across each gene, with individual haplotypes being tested for association with schizophrenia. In the second approach, further fine mapping was undertaken by genotyping an evenly spaced SNP map across the entire 30 Mb region and the data tested for both linkage and association in the original family collection. Data from both of these strategies will be presented and all SNP data is combined to provide a first generation haplotype map of the region.

**Attempt To Maximize Efficiency In Screening Mentally Retarded Males For MECP2 Mutations: Microcephaly, Spasticity and Seizures As Clinical Criteria And Screening For The A140V Mutation.** *F. Lobo-Menendez, R.C. Michaelis, F. Annese, C. Skinner, C.E. Schwartz.* JC Self Research Institute, Greenwood Genetic Center, Greenwood, SC.

The methyl-CpG binding protein 2 (MECP2) gene was first identified as the gene responsible for Rett syndrome (RTT), a neurobehavioral disorder that primarily affects females. RTT is characterized by a 6-12 month period of normal development, followed by the regression of motor, cognitive and language skills, deceleration of head growth leading to acquired microcephaly, onset of seizures and the emergence of autistic behaviors. While MECP2 mutations were originally thought to be lethal in males, recent studies have documented MECP2 mutations in males with mental retardation (MR). The spectrum of phenotypic effects of MECP2 mutations in males ranges from mild nonsyndromic MR to severe encephalopathy and death during infancy, and patients exhibit variable combinations of other phenotypic abnormalities as well.

These findings have prompted efforts to determine the most efficient manner in which to screen mentally retarded males for MECP2 mutations. One study suggested that the most common MECP2 mutation, the A140V substitution, may be common enough in mentally retarded males to warrant routine screening of all males with MR for the A140V mutation. We have tested this suggestion in our population of mentally retarded males, and found no A140V mutations in 525 patients tested. In addition, we selected patients from our MR population who also had microcephaly, seizures or spasticity, to determine the frequency of MECP2 mutations in patients with MR and these additional features. A number of missense variants have been found in these patients. Family members and unrelated controls are being tested to confirm the pathogenic nature of these variants. However, the majority of these variants appear to be benign polymorphisms, and MECP2 mutations appear to be rare in our selected patient population.

**Impact of macro nutrient content of low calory diet on human adipose tissue gene expression.** *I. Dahlman<sup>1</sup>, K. Linder<sup>2</sup>, J. Liden<sup>3</sup>, E. Arvidsson<sup>1</sup>, P. Arner<sup>1</sup>.* 1) Dept Medicine, Karolinska Inst, Huddinge Univ Hosp, Stockholm, Sweden; 2) Center for Molecular Medicine, Karolinska Inst; 3) Center for Biotechnology, Karolinska Inst.

Calorie restriction results in weight loss, however to what extent diet composition, i.e. the relative content of different nutrients, control degree and mechanisms of weight loss are unknown. The aim of this project was to compare the outcome of a high and a low fat diet on the gene expression profile in adipose tissue. Age and weight matched women were randomly selected for high fat (N = 13) or low fat (N = 10) eight week low calory diet. Before initiation of the program and after its termination patients were clinically examined and an abdominal subcutaneous fat biopsy obtained for measurement of fat cell size and gene expression using Affymetrix microarrays containing 8500 of the best characterized human genes. Overall, both groups responded similar with regard to weight loss, blood chemistry phenotypes and decrease in fat cell size. The number of genes altering their expression following diet was relatively small. 40 genes with a relative and significant change of > 25 % in adipose tissue expression before vs after the weight reduction program were detected. There was no diet-specific impact on gene expression, i.e. the same genes were affected in both groups. There was a marked reduction in expression of a number of genes involved in lipid metabolism. In addition, genes regulating carbohydrate metabolism were affected but to a lesser extent. Of interest, the expression of steryl coenzyme A desaturase (SCD) was downregulated 50 %; in response to weight reduction. Mice with a targeted disruption of SCD are protected against obesity. Expression of certain obesity candidate genes correlated with relative weight loss, e.g. NPY Y1 receptor. More detailed studies of SCD and other identified genes are ongoing. We conclude that SCD and other identified genes in lipid metabolism may control important pathways in human body weight regulation and thus represent new therapeutic targets.

**Effects of Activin on Somatic Growth in B Knock-in Mice.** *L. Li<sup>1</sup>, C. Tang<sup>1</sup>, J. Shen<sup>1</sup>, C. Brown<sup>1,2</sup>*. 1) Molecular and Human Genetics; 2) Pediatrics, Baylor College of Medicine, Houston, TX.

To better understand the molecular mechanisms of growth deficiency so that more directed therapies can be developed, we study activins, which are members of the transforming growth factor beta (TGF-) superfamily. They are important for many different biological processes including normal growth and development. Mice that are completely missing one activin family member, activin A, develop cleft palate, fail to form teeth, and do not grow whiskers. Secondary to these and other defects, the mice die shortly after birth. Because of this neonatal lethality, our laboratory has introduced a hypomorphic (activin B knock-in) allele at the activin A locus (BK). Although these mice develop normal craniofacial structures, they grow more slowly than normal, influenced dramatically by the dose of the BK allele. In this study, we are trying to understand the mechanism(s) by which BK mice fail to thrive by carefully measuring growth response to caloric density, metabolic rates, and intake, and also assaying specific analytes that influence somatic growth. We find that high fat diets (HFD) cannot make BK/BK mice grow as well as WT or BK/+ mice on the same diet. However, BK/BK mice on HFD exhibit catch up growth, approximating the weight curves of WT and BK/+ mice maintained on regular diet. Plasma growth hormone levels in 42 day old BK/BK mice on regular diet were not different from controls. In contrast, serum IGF-1 levels of BK/BK mice were significantly less than controls. Major urinary protein levels, an indicator of normal GH secretory patterning, were normal in both male and female BK/BK mice relative to control mice. IGF and GH treatment studies, insulin and glucose tolerance studies, histological and other analyses are in progress. Our results suggest that activin signaling influences many physiological processes, potentially mediated through IGF-1, but not substantially influenced by growth hormone production or release. Additionally, possible reasons for the growth delay in BK/BK mice include reduced intake or increased metabolic rates, as increasing caloric density improves their growth.

**Decreased Mitochondrial tRNA<sup>Lys</sup> Steady-State Levels and Aminoacylation are Associated with the Pathogenic G8313A Mitochondrial DNA Mutation.** *S.R. Bacman, D.P. Atencio, C.T. Moraes.* Neurology, University of Miami, Miami, FL.

Mutations in human mitochondrial tRNA genes cause a number of multisystemic disorders. A G8313A transition in the mitochondrial tRNA<sup>Lys</sup> gene has been associated with a childhood syndrome characterized by gastrointestinal system involvement and encephaloneuropathy. We have used transmitochondrial cybrid clones harboring patient-derived mitochondrial DNA with the G8313A mutation for the study of the molecular pathogenesis. Our results showed that mutant mitochondrial cybrids with 82%, 97% and 100% mutated mtDNA had a severe respiratory chain enzymes activity with a markedly defect in Complex II+III and Complex IV (up to 95% when compared to the homoplasmic wild type). Steady state levels of COX II, a subunit of cytochrome c oxidase and ND1, a subunit of complex I (both mtDNA-encoded subunits), were markedly reduced in the mutant cybrid cell lines. In contrast, a truncated version of COX I was surprisingly stable in mutant cells, as shown by western blot. Mutant cybrids also showed a large number of what appeared to be truncated polypeptides in the mitochondrial protein synthesis experiment, whereas most of the normally-sized bands were decreased or absent. Acid northern blot assays showed that the G8313A mutation affects both steady-state levels and aminoacylation efficiency of the tRNA<sup>Lys</sup>. These observations suggest decrease in functional tRNA<sup>Lys</sup> levels underlie the pathogenesis of the mitochondrial G8313A mutation. They also showed that non-functional truncated polypeptides could be stable in the absence of the holo-complex IV.

**Mitochondria haplogroup B4b1 is associated with an increased risk of familial cholelithiasis in Chinese.** *Z. Niu*<sup>1</sup>, *B. Wen*<sup>2</sup>, *T. Han*<sup>3</sup>, *J. Qin*<sup>3</sup>, *W. Yuan*<sup>1</sup>, *S. Zhang*<sup>3</sup>, *L. Jin*<sup>1,2,4</sup>, *W. Huang*<sup>1</sup>. 1) Chinese Natl Hum Genome Ctr, Shanghai, Shanghai, China; 2) School of Life Sciences, Fudan University; 3) Rui Jin Hospital affiliated to Shanghai Second Medical University; 4) Center for Genome Information, University of Cincinnati.

Cholelithiasis is a condition with strong family predisposition. The incidence of cholelithiasis in China is about 7-10%. The supersaturation of cholesterol in bile leads to its crystallization and formation of a nidus for stone. Cytochrome C oxidase complex is composed of eight subunits including three (COX1, COX2, COX3) that are coded by mitochondrion genome. It plays a crucial role in beta-oxidation and oxidative phosphorylation in fat and steroid metabolism. Therefore, it is reasonable to be explored although the association between haplotypes of mitochondria and familial cholelithiasis has never been reported. In this study, we sequenced the genes of three mitochondrial COX subunits in 101 patients from Shanghai and revealed many SNPs (62 in COX1, 29 in COX2 and 35 in COX3). As a result, three SNPs in COX1 (6023 G to A; 6216 T to C; 6413 T to C) were found to have higher allele frequencies and significant association was only observed at 6413 T to C ( $p < 0.01$ ) and 6023 G to A ( $P < 0.05$ ) with the 535 controls from Shanghai. Furthermore, we sequenced the HVS1 region in all patients and 535 controls from Shanghai. An East Asian specific haplogroup B4b1, which is characterized by 16136C-16189C-16217C motif, showed a strong association with an increased risk of familial cholelithiasis in Chinese population (B4b1,  $p < 0.0001$ ; B4b1a,  $p < 0.00001$ ). The relative risk (RR) of cholelithiasis for individuals carrying B4b1 and B4b1a are 6.778 and 13.049, respectively. To identify the molecular lesion that elevates the risk of this disease in B4b1 haplogroup, we sequenced the complete mtDNA genome in six patients with haplogroup B4b1. Several SNPs which haven't been reported before were found in these patients. The functional role of these potential variants in B4b1 haplogroup responsible for cholelithiasis susceptibility is yet to be found. This is the first observation of a significant association of mtDNA haplotype with cholelithiasis.

**Contribution of mitochondrial genome to hypertension.** *F. Schwartz*<sup>1</sup>, *A. Duka*<sup>1</sup>, *J. Cui*<sup>1</sup>, *H. Gavras*<sup>1</sup>, *F. Sun*<sup>2</sup>. 1) Department of Medicine, Boston University School of Medicine, Boston, MA; 2) University of Southern California, Los Angeles, CA.

Genetic studies of essential hypertension (HTN), a polygenic, multifactorial and highly heterogeneous disorder of unknown etiology, thus far produced inconsistent and often contradictory findings. As the primary focus of molecular genetic analysis is on the nuclear genes, the role of the mitochondrial genome in HTN remains unexplored. Recently, we reported excess maternal transmission in familial HTN, suggesting a possible mitochondrial involvement. In an effort to assess the contribution of the mitochondrial genes to HTN, we initiated a systematic and extended screening of hypertensive individuals to identify potentially pathogenic mtDNA mutations. We applied our newly developed novel class of tests for the detection of mitochondrial mutation involvement in complex diseases (Sun et al. 2003) to the hypertension data set from 350 Caucasian and 98 African American pedigrees ascertained at HTN clinics associated with Boston Medical Center, and identified families with a likely mitochondrial involvement. Thus far, we analyzed the sequence of the entire mitochondrial genome of probands from 19 such pedigrees. They include 10 African Americans, 6 American Caucasians and 3 Greek Caucasians from our collaborating site in Greece. Comparison with the consensus Cambridge sequence revealed multiple base changes in all hypertensive individuals examined, some of which were observed in several individuals (such as 4216C within the ND1 gene and 10398G within the ND3 gene); some appear to be haplogroup-specific (such as 3308C in the ND1 gene and 5655C in the tRNA-Ala gene, both detected only on the L1b1 background); and some found only in one given family (these include many tRNA variants). These data, combined with sequence analysis of additional pedigrees, will serve as a starting point for a large-scale case-control association studies.

**Assessing the role of the mitochondrial genome in type 2 diabetes mellitus and related traits in Finns.** *E.C. Peck<sup>1</sup>, K.L. Mohlke<sup>1</sup>, A.U. Jackson<sup>2</sup>, Y. Suh<sup>1</sup>, L.J. Scott<sup>2</sup>, J. Tuomilehto<sup>3</sup>, R.N. Bergman<sup>4</sup>, M. Boehnke<sup>2</sup>, F.S. Collins<sup>1</sup>, FUSION Study Group.* 1) NHGRI/NIH, Bethesda, MD; 2) U. Michigan, Ann Arbor, MI; 3) National Public Health Institute, Helsinki, Finland; 4) U. Southern California, Los Angeles, CA.

Type 2 diabetes mellitus (T2DM), a complex gene disorder, accounts for approximately 90% of diabetes cases. T2DM has strong genetic and environmental components and is characterized by insulin resistance as well as pancreatic beta cell dysfunction. Since mitochondria play an integral role in ATP production in cells and are involved in glucose metabolism and insulin secretion, variants in the mitochondrial genome are plausible candidates for contributing to diabetes susceptibility. In our study of 793 Finnish families ascertained for T2DM, probands reported affected mothers three times more frequently than they reported affected fathers. To assess whether this apparent excess maternal transmission can be explained by mitochondrial variants, we selected single nucleotide polymorphisms (SNPs) based on phylogenetic networks. We selected 15 SNPs that define the major haplogroups and 18 that subdivide groups H and U, which are common in Finns. We genotyped the SNPs on one T2DM case per family and unaffected controls using primer extension MALDI-TOF mass spectrometry. We were able to assign haplogroups to 789 cases and 414 controls; in addition, we assigned the maternal haplogroup to 480 offspring of genotyped females. We evaluated association with both affected status and 8 to 14 diabetes-related traits for each of the cases, controls, and offspring. One of these traits was glucose effectiveness, which is the ability of glucose to enhance glucose disposal independent of an increment in insulin. The strongest evidence for phenotypic association was reduced glucose effectiveness in offspring from haplogroup V (p-value = 0.0003). No haplogroup, however, showed notable association with T2DM affected status (best p-value = 0.081 for haplogroup J). This study suggests that mitochondrial variants may play a modest role in glucose metabolism in the Finnish population.

**A Mouse Model of Bardet-Biedl Syndrome type 2.** V.C. Sheffield<sup>1,2</sup>, D.Y. Nishimura<sup>1</sup>, C. Searby<sup>1,2</sup>, K. Mykytyn<sup>1,2</sup>, M. Andrews<sup>1,2</sup>, C. Eastman<sup>4</sup>, B. Yang<sup>3</sup>, E.M. Stone<sup>2,4</sup>. 1) Dept of Pediatrics, University of Iowa, Iowa City, IA; 2) Howard Hughes Medical Institute; 3) Dept of Obstetrics/Gynecology, University of Iowa, Iowa City, IA; 4) Dept of Ophthalmology, University of Iowa, Iowa City, IA.

Bardet-Biedl syndrome (BBS) is a genetically heterogeneous disorder with the clinical features of obesity, pigmentary retinopathy, polydactyly, hypogenitalism, mental retardation and renal anomalies. Secondary features include diabetes mellitus, congenital heart disease and hypertension. Five BBS genes have been identified on human chromosomes 11 (*BBS1*), 16 (*BBS2*), 15 (*BBS4*), 20 (*BBS6*) and 4 (*BBS7*). Two additional loci have been mapped to chromosomes 3p13-p12 (*BBS3*) and 2q31 (*BBS5*). The BBS genes have a wide pattern of tissue expression and except for *BBS6*, which has similarity to a *Thermoplasma acidophilum* chaperonin, the genes are novel. Although historically thought to be an autosomal recessive disorder, complex inheritance of BBS has been proposed based in part on a family with two discordant siblings homozygous for a nonsense mutation in the *BBS2* gene. In order to further study the genetics and pathophysiology of BBS we are developing animal models of this disorder. We have successfully targeted the *Bbs2* gene in mouse embryonic stem cells resulting in the deletion of exons 5 to 13. Mice homozygous for this knockout mutation (*Bbs2*<sup>-/-</sup>) appear normal at birth but have been obtained at less than the expected frequency (4/48, p<0.005). Electroretinography (ERG) of 11 week old *Bbs2*<sup>-/-</sup> mice revealed a 90% reduction in b-wave amplitude indicating that these mice have the retinopathy component of the human phenotype. Additional studies are underway to further define the *BBS2* phenotype in mice and to determine whether the BBS genes demonstrate genetic interaction.

**Tuberous Sclerosis 2 (TSC2) Gene Variants In Patients With Autism And Seizures.** *R.C. Michaelis<sup>1</sup>, C.E. Steiner<sup>2</sup>, F. Lobo-Menendez<sup>1</sup>, C. Skinner<sup>1</sup>, C.M. Wright<sup>3</sup>, R.J. Schroer<sup>1</sup>.* 1) JC Self Research Inst, Greenwood Genetic Ctr, Greenwood, SC; 2) University of Sao Paulo-Campinas, Campinas, Brazil; 3) Presbyterian College, Clinton, SC.

Despite ample evidence that genetic factors play an important role in the etiology of autism, the genetic causes of autism have been identified in very few cases. Linkage analyses have implicated 16p13 as a potential location for an autism genes. Among the genes in this region is the tuberous sclerosis 2 (TSC2) gene, which encodes the GTPase activating protein tuberin. Tuberin is thought to play an important role in neuronal migration, proliferation and differentiation. TSC2 mutations account for 50-60% of TSC cases. Over 90% of TSC patients demonstrate evidence of CNS involvement, including brain malformations, seizures and/or mental retardation.

The relationship between autism and TSC is particularly intriguing, because of a surprisingly high level of comorbidity between the two disorders. Autism has been estimated to occur in 17-68% of TSC patients. Conversely, TSC has been reported in 1-4% of patients with autism, and 8-14% of patients with autism and seizures. Little is known regarding the pathophysiological mechanism by which autism and TSC may be related, however. Some studies have suggested that autism is associated with tubers in the temporal lobe in TSC patients, but others find no association between autism and tuber location.

The TSC2 gene was screened for mutations in 38 patients with autism and seizures plus 3 patients with autism and TSC. The spectrum of variants found includes at least one nonsense and several missense variants. However, segregation analyses within families suggest that many of these TSC2 mutations, even the nonsense mutation, are not sufficient to produce autism, but may constitute a component of a "multiple-hit" scenario by which autism is produced by dysfunction in several interacting genes and/or environmental factors.

**Apolipoprotein L6 (ApoL6), expression up-regulated in schizophrenia, is a novel BH3-only pro-apoptotic protein.**

Z. Liu<sup>1</sup>, Z. Jiang<sup>2</sup>, H. Lu<sup>1</sup>, C.A. HU<sup>1</sup>. 1) Dept. Biochem. Mol. Biol., Univ. New Mexico Health Sciences Center, Albuquerque, NM; 2) National Center for Genome Resources, Santa Fe, NM.

Apoptosis is a complex and highly regulated physiological process that eliminates unwanted cells. Dysregulation of apoptosis is involved in many human diseases including neuro-degenerative disorders, cancer and atherosclerosis. Members of the Bcl-2 family play pivotal roles in regulating apoptotic pathways in which they are either anti-apoptotic (e.g., Bcl-2) or pro-apoptotic (e.g., PUMA). They possess one to four conserved protein sequences Bcl-2-Homology (BH) domains, designated BH1, BH2, BH3, and BH4. The BH3 domain is the only one conserved in all Bcl-2 family members and is present in pro-apoptotic "BH3-only" proteins. The BH3 domain plays an important role in protein-protein interactions and in regulating dimerization of the Bcl-2 family members in apoptosis. The goal of this study was to identify and characterize novel BH3-containing proteins from the human proteome. The completion of the Human Genome Project and the availability of various public databases allowed us to utilize the bioinformatic data-mining approach to conduct databases search. We employed a 9 amino-acid BH3 domain consensus sequence to screen public databases and identified three novel human BH3-containing proteins, one of which, apolipoprotein L6 (ApoL6), is a 343 amino-acid cytoplasmic protein. ApoL proteins belong to a newly identified, high density lipoprotein family comprised of six members, L1 to L6. The genes encoding ApoLs are localized to chromosome 22q12-13, a high-susceptibility locus for schizophrenia. ApoL1, L2, L4 and L6 have been shown to be significantly up-regulated in schizophrenia (1). To investigate if ApoL6 is a *Boni fide* BH3-only apoptotic protein, we cloned and functionally expressed the full-length human ApoL6 cDNA in a Tet-off inducible cellular model and showed that the overexpression of ApoL6 induces mitochondria-mediated apoptosis-- phenotypes include release of cytochrome c, activation of caspase 9 and nuclear fragmentation. In summary, ApoL6 is a BH3-only pro-apoptotic protein and may be involved in the etiology of schizophrenia.(1) Mimmack et al. (2002) PNAS 99, 4680.

**Candidate genes for myopia - The role of opticin, fibromodulin, PRELP and lumican genes.** *M. Majava<sup>1</sup>, M. Männikkö<sup>1</sup>, P. Bishop<sup>2</sup>, L. Ala-Kokko<sup>1</sup>.* 1) Department of Medical Biochemistry and Molecular Biology, University of Oulu, Finland; 2) Wellcome Trust Center for Cell-Matrix Research, School of Biological Sciences, and Research Group in Eye & Vision Sciences, The Medical School of University of Manchester, UK.

Myopia is the most common eye disease in Western Europe and USA with the incidence of 10-25 %. The environment effects on development of low myopia (physiological), but a strong genetic component has been shown for intermediate and high (pathological) myopia. Three loci have been mapped: Xq (MYP1), 18p (MYP2), and 12q21-23 (MYP3). Human vitreous gel is acellular and highly hydrated extracellular matrix. The gel structure is maintained by a dilute meshwork of collagen fibrils (types II, V/XI and IX). In vitreous degeneration the gel-like structure becomes more liquid as the collagen fibrils collapse. Opticin, fibromodulin, PRELP and lumican belong to a family of extracellular matrix small leucine-rich repeat proteins (SLRP) non-covalently bound to the surface of collagen fibrils. The SLRP proteins have been shown to regulate collagen fibril diameter and spacing through the binding. Opticin is the predominant leucine-rich repeat protein found in vitreous. PRELP, fibromodulin and lumican are expressed in the human sclera. In humans scleral thinning and changes in the collagen fibril diameter are associated with the development of high myopia. In mice null mutations in one or both alleles of the lumican gene result in defects in scleral collagen fibril formation. The genes for human opticin, PRELP and fibromodulin are present in a gene cluster on 1q32. The lumican gene locates in a SLRP cluster at 12q21-23. In the present study a heteroduplex analysis (CSGE) and subsequent sequencing of the exons containing heteroduplexes was performed for opticin, fibromodulin, PRELP, and lumican genes in 86 myopic (> 6 D) patients with family history of the disease. Total of six changes were found, four in lumican and two in fibromodulin gene that were not found in control set of matched population. The results suggest that changes in these genes may be part of the predisposing genetic factors for intermediate and high myopia.

**Assessment cocaine and amphetamine-regulated transcript (CART) gene polymorphism in Korean Alcoholism.**

*M.S. Hong<sup>1</sup>, H.J. Lee<sup>1</sup>, S.Y. Jin<sup>1</sup>, Z.W. Kim<sup>2</sup>, H.J. Park<sup>2</sup>, J.H. Chung<sup>1</sup>.* 1) Department of pharmacology, College of Medicine, Kyung Hee University, Seoul, Republic of Korean; 2) Kowhang Medical Research Institute College of Medicine, Kyung Hee University, Seoul, Republic of Korea.

Cocaine and amphetamine-regulated transcript (CART) peptides are novel gene peptides on putative brain-gut neurotransmitters and co-transmitters that probably have a role in drug abuse, the control of feeding behavior, sensory processing, stress and development. We examined whether single nucleotide polymorphism of the CART gene is associated with a risk for alcohol dependence. Subjects were 100 Korean male alcoholics and 199 age-matched male controls. We performed the distribution of genotypes and frequencies of the AvaII polymorphism. There was significant difference in the genotype distribution of polymorphism between Korean male alcoholics and control group ( $P < 0.05$ ). In further study, more large number of subjects would be required to study whether CART polymorphism is associated with alcohol dependence.

**Impact of IL-1 receptor antagonist gene polymorphism on schizophrenia and bipolar disorder.** *S. Kim<sup>1</sup>, H.J. Lee<sup>2</sup>, J.W. Kim<sup>2</sup>, H.J. Park<sup>2</sup>, S.H. Yoon<sup>3</sup>, M.K. Kim<sup>4</sup>, S.Y. Jin<sup>2</sup>, M.S. Hong<sup>2</sup>, S.V. Yin<sup>5</sup>, D.H. Shin<sup>6</sup>, J. Chung<sup>2</sup>.* 1) College of Life Science & Biotechnology, Korea University, Seoul, Republic of Korea; 2) Kowhang Medical Research Institute, Department of Pharmacology, College of Medicine, Kyung Hee University, Seoul, Republic of Korea; 3) College of Pharmacy, Kyung Hee University, Seoul, Republic of Korea; 4) Seoul National Mental Hospital, Seoul, Republic of Korea; 5) Department of Pharmacology, College of Medicine, Kangwon University, Chunchon, Republic of Korea; 6) Department of Preventive Medicine, School of Medicine, Keimyung University, Daegu, Republic of Korea.

The association between an abnormal immune system and schizophrenia or bipolar disorder was suggested by several studies, and cytokine may be important in those disorder. Especially, the level of proinflammatory cytokines was varied on schizophrenia patients. The IL-1 receptor antagonist(IL-1RA) inhibits IL-1 by competing for receptor binding. In this study, we performed association study of VNTR polymorphism of the IL-1RA gene on schizophrenia and bipolar disorder. A total of 269 patients with schizophrenia, 83 patients with bipolar disorder and 297 Korean healthy control subjects were included. There were a significant difference in the IL-1RA polymorphism between schizophrenia group and controls, and the carriage of IL1RN\*2 allele was associated with the increase risk to schizophrenia. However, there was no association with bipolar disorder with IL-1RA variant. Our results suggest that cytokine alteration in schizophrenia may be a genetic etiology, and IL-1RA could be a candidate gene for susceptibility to schizophrenia.

**Melanocortin-4-Receptor Gene Mutations in Morbidly Obese Finnish Children and Adults.** *K. Valli-Jaakola<sup>1</sup>, M. Lipsanen-Nyman<sup>2</sup>, L. Oksanen<sup>1</sup>, A.N. Hollenberg<sup>4</sup>, K. Kontula<sup>1</sup>, C. Bjørnbæk<sup>4</sup>, C. Schalin-Jäntti<sup>1,3</sup>.* 1) Department of Medicine, University of Helsinki, Finland; 2) The Hospital for Children and Adolescents, Helsinki University Hospital, Finland; 3) Department of Endocrinology, Helsinki University Hospital, Finland; 4) Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA.

Mutations in the melanocortin-4-receptor (MC4R) gene have been shown to be the most common known monogenic cause of severe early-onset obesity. The aim of the present study was to investigate if morbid obesity in Finns could be explained by mutations in the MC4R gene. We screened the MC4R gene by denaturing high performance liquid chromatography and direct sequencing in 56 children with severe early-onset obesity (relative weight for height +70% before age 10, 29 females / 27 males) and 252 morbidly obese adults (BMI 40 kg/m<sup>2</sup>, 182 females / 70 males). The prevalence of the mutations detected was determined in a background population of 321 healthy blood donors. In one child we identified a pathogenic mutation (S127L) causing severe early-onset obesity. We also detected one novel (I226T) and three previously described (V103I, T112M, I125L) polymorphisms on the coding region of the gene. In addition, two new variations (-439delGC and 1059C>T) outside the coding region of the MC4R gene were identified. The signaling properties of the mutant receptors were studied, in addition to stimulation with the agonist -melanocyte stimulating hormone (-MSH), with desacetyl--MSH, -MSH, <sub>1</sub>-MSH and the antagonist Agouti Related Protein (AGRP). In vitro functional studies of the variants T112M, S127L and I226T demonstrated a severely impaired signaling by the S127L receptor, strongly supporting a pathogenic role for this mutation. The impaired function was due to reduced activation and not a defect of protein expression or transport to the cell membrane. In conclusion, one pathogenic mutation was found in a child with severe early-onset obesity. MC4R sequence variations are found in the Finnish population with similar frequencies as described in other populations.

**Parkin mutation analysis in clinic patients with early-onset Parkinson's disease.** *P. Poorkaj*<sup>1, 2</sup>, *J. Nutt*<sup>3</sup>, *D. James*<sup>3, 4</sup>, *E. Steinbart*<sup>1</sup>, *S. Gancher*<sup>5</sup>, *T. Bird*<sup>1, 2</sup>, *G. Schellenberg*<sup>1, 2</sup>, *H. Payami*<sup>3, 4</sup>. 1) Dept of Medicine, Univ of Washington, Seattle; 2) GRECC, VA, Seattle; 3) Dept of Neurology, OHSU, Portland, OR; 4) Wadsworth Center, Albany, NY; 5) Kaiser, Portland, OR.

Once believed to be non-genetic, Parkinson's disease (PD) has proved to be a heterogeneous disorder with a significant genetic component. In the past seven years, more than ten loci have been linked to PD, four of which (a-syn, UCHL1, parkin and DJ-1) have been identified and mutations characterized. parkin is the most common genetic cause of PD. parkin mutations are found predominantly in early-onset PD (at or before age 40) and are presumed to be recessive. It has been proposed that parkin testing should be a part of clinical work up for young onset PD. The purpose of this study was to assess parkin mutation frequency in a clinic setting, correlate genotype and phenotype, and evaluate the current justification for clinical parkin testing. Patients were selected from a movement disorder clinic based on diagnosis of PD (N=442) and early-onset (39/442). parkin was genotyped by sequence and dosage analysis for all exons. Relatives and controls were screened for mutations. Mutations were found in 7/39 patients and 0/96 controls. Two were compound heterozygous; 5 were heterozygous. Mutations included deletions in E2, E3, E8, duplications in E2-4, E9, and P437L. 70% of mutations were detected by dosage analysis. A novel substitution (R402W) was found in one patient and one control. The phenotype of parkin-positive patients was remarkably similar to patients without parkin mutations. In conclusion, parkin mutations are common in early-onset PD patients (18%). parkin cases cannot be distinguished on the basis of clinical features and require genetic testing. parkin testing is critical for research, but its wide application for diagnosis and counseling is premature. parkin mutations are presumed to be recessive, yet 70% of parkin cases were heterozygous. It is unclear if heterozygous mutations are pathogenic, or another mutation is embedded in parkin. parkin-based diagnosis and counseling requires a better understanding of the mode of inheritance, penetrance and carrier frequencies.

**Growth hormone receptor gene and idiopathic short stature.** *M. Taro, C. La Rosa, A. Citana, M. Baldi, R. Gastaldi, C. Traggiai, C. Bellini, E. Bonioli.* Pediatric Department , State University of Genova, Genova, Italy.

Although several pathological processes can lead to impaired linear growth, many short children do not fit into any clearly defined diagnostic category. These patients are usually classified as having idiopathic short stature (ISS). Although this is an unsatisfactory description, the term ISS is commonly used to describe children with: height < 2 standard deviations (SD); bone age delay > 2 SD; height speed < 25 centile; normal plasma GH level; no evidence of chronic physical or psychological illness. Recently partial Growth Hormon Insensitivity (GHI) has been reported in patients with ISS. GHI is identified as the inability to respond in a normal way to GH. The genetic form of GHI is historically known as Laron Syndrome, it consists of a severe hypostaturalism, and it is associated to a well known typical phenotype. It is due to homozygous mutations of GH receptor gene, resulting in a complete GH insensitivity. Recently, it has been appreciated that abnormalities of the growth hormone receptor (GHR) gene may be associated with relatively modest reduction of height in absence of the classical phenotype. It has also been proposed that partial GHI due to heterozygous mutations of the GHR may account for some cases of short stature previously classified as idiopathic (ISS). We studied GHR gene in 37 ISS patients referred to our Clinic and in 50 controls of normal stature, matched for age, sex and geographical area. Sequence analysis of PCR products of GHR gene revealed in 1 patient a novel heterozygous base pair change c485T>C in exon 6, resulting in mutation V162A. In 13 patients and in 26 controls we found the previously described polymorphism c558A>G, resulting in a silent mutation G186G. In 1 patient a novel polymorphism c336T>C in exon 5 was found, resulting in the silent mutation C112C. Our results suggest that in some patients there may be a correlation between ISS phenotype and GHR gene mutations.

**Analysis of Methyl Binding Domain (MBD) genes in autism risk.** *R. Rabionet<sup>1</sup>, J. Jaworski<sup>1</sup>, M.L. Cuccaro<sup>1</sup>, J.L. Benton<sup>1</sup>, S. Walters<sup>1</sup>, H.H. Wright<sup>2</sup>, R.K. Abramson<sup>2</sup>, G.R. DeLong<sup>1</sup>, J.R. Gilbert<sup>1</sup>, M.A. Pericak-Vance<sup>1</sup>.* 1) Duke University Medical Center, Durham, NC; 2) University of South Carolina, Columbia, SC.

Mutations in the methyl-CpG-binding protein 2 (MECP2) gene have been described to cause Rett syndrome and other X-linked mental retardation syndromes. Some cases of Rett syndrome patients have been reported to display autistic features. Recently, mutations in MECP2 have also been reported in autistic disorder cases. The involvement of MECP2 in autism suggests that other genes from the same family might also be involved in autism susceptibility. MECP2 shares the ability to bind to methylated DNA with four other proteins that have a similar Methyl-Binding Domain (MBD), and that are named MBD1-4. The genes encoding these proteins are located on chromosomes 18q21 (MBD1 and 2), 19p13 (MBD3) and 3q21 (MBD4). Preliminary analysis of MBD4 by DHPLC and sequencing in a dataset of 238 autistic families (both multiplex and sporadic (no family history of autism)), and in 32 control samples identified two intronic and 4 exonic variations. The intronic changes consisted of a C to G change at IVS2 -46 and a T to G change at IVS3+50, and were identified in both the case and control datasets. All the exonic variations identified caused amino acid changes. Three of them were identified in multiple cases as well as in controls: a G to T change at position 162 of the coding sequence caused a Met to Ile change, a G to A at 817 caused a Ala to Tre variation, and a T to C change at 1073 caused a Ile to Tre substitution. The fourth coding variant was only identified in two cases, and no controls, and consists of an A to G change at position 1399, causing a substitution of Asn to Ser. The individuals carrying this variant are sporadic autism families thus, screening of additional controls will be necessary to assess the significance of this variant in autism susceptibility. Complete analysis of MBD1-4 genes and their association with autism genetic risk will be presented.

**Variation in the Calpain-10 Gene (CAPN10) is Associated with Age-at-Diagnosis of Type 2 Diabetes in Japanese Subjects.** *N. Iwasaki<sup>1</sup>, Y. Horikawa<sup>2</sup>, T. Nakamura<sup>3</sup>, T. Tsuchiya<sup>4</sup>, M. Ogata<sup>1</sup>, N. Kamatani<sup>3</sup>, N.J. Cox<sup>5</sup>, Y. Iwamoto<sup>1</sup>.*  
1) Diabetes Ctr, Tokyo Women's Medical Univ, Tokyo, Japan; 2) Dept of Cell Biology, Gunma Univ, Maebashi, Japan; 3) Dept of Statistical Genetics, Institute of Rheumatology, Tokyo Women's Medical Univ, Tokyo, Japan; 4) Dept of Biochemistry and Molecular Biology, Univ of Chicago, Chicago, IL; 5) Dept of Human Genetics, Univ of Chicago, Chicago, IL.

Variation in the gene encoding the ubiquitously expressed non-lysosomal cysteine protease calpain-10 has been associated with risk of type 2 diabetes and with diabetes-related traits. Risk is determined by both specific polymorphisms and haplotypes. We have typed three haplotype-tagging SNPs in the calpain-10 gene (SNP-43, -19 and -63) in 205 unrelated Japanese subjects with type 2 diabetes and in 208 controls with normal glucose tolerance. There was no significance difference in frequency of SNP-43 and -19 between cases and controls but the SNP-63 T-allele was present at a significantly higher frequency in cases compared to controls (0.34 and 0.27, respectively,  $P = 0.042$ ). SNP-63 also showed association with area under the curve (AUC) glucose 0-120 min during an oral glucose tolerance test in 281 non-diabetic subjects ( $P = 0.01$ ). Patients with the SNP-63 T/T genotype tended to have a later age-at-diagnosis than those with other genotypes ( $P = 0.075$ ). An effect of calpain-10 genotype on age-at-diagnosis has not been previously observed and we therefore examined this association in a second independent group of patients ( $n = 151$ ). SNP-63 was not associated with type 2 diabetes in this group. However, individuals with the T/T genotype also tended to have a later age-at-diagnosis of type 2 diabetes ( $P = 0.069$ ). A combined analysis of the two patient groups showed a significant difference in age-at-diagnosis of type 2 diabetes: [C/C + C/T] vs T/T; 47.10.7 vs 51.72.0 years, respectively;  $P = 0.037$ . The results suggest that calpain-10 may be a risk factor for type 2 diabetes in Japanese and variation in the calpain-10 gene may modify the age-at-onset of diabetes.

**The contribution of corneodesmosin to androgenetic alopecia.** *R.C. Betz<sup>1</sup>, S. Hanneken<sup>2</sup>, T.G. Schultze<sup>3</sup>, G.M. Kukuk<sup>4</sup>, S. Ritzmann<sup>2</sup>, A.M. Hillmer<sup>4</sup>, C. Metzner<sup>2</sup>, S. Cichon<sup>1</sup>, T. Ruzicka<sup>2</sup>, P. Propping<sup>4</sup>, R. Kruse<sup>2</sup>, M.M. Noethen<sup>1</sup>.* 1) Dept. of Medical Genetics, University of Antwerp, Antwerp, Belgium; 2) Dept. of Dermatology, University of Duesseldorf, Duesseldorf, Germany; 3) Central Institute of Mental Health, Mannheim, Germany; 4) Institute of Human Genetics, University of Bonn, Bonn, Germany.

Androgenetic alopecia (AGA, male pattern baldness) is the most common form of hair loss in men and women. Significant AGA affects well over one half of the adult male population. It is characterized by a loss of hair from the scalp that follows a defined pattern. The development of AGA is androgen-dependent and of genetic origin. To date, a very limited number of studies have been performed concerning the molecular genetic basis of AGA.

Recently, we identified corneodesmosin (CDSN) as the responsible gene for hypotrichosis simplex of the scalp (HSS). HSS is an autosomal dominant form of isolated alopecia causing almost complete loss of scalp hair, with onset in childhood. Interestingly, among the known single gene hair disorders, the phenotype in HSS resembles the phenotype in androgenetic alopecia. Furthermore, the histological picture of scalp biopsies from patients with HSS has many features of androgenetic alopecia such as miniaturized follicles of the vellus type and absence of scarring.

Following the identification of the gene for HSS, we thoroughly examined CDSN for a possible contribution to the development of androgenetic alopecia in a sample of 168 trios, 177 single probands and 111 controls. We selected 21 intragenic variants, including 12 SNPs causing amino acid exchanges. Preliminary results at the level of individual polymorphisms in CDSN did not show any significant association with AGA. Further statistical analyses are currently underway.

Program Nr: 2177 from 2003 ASHG Annual Meeting

**Bsm I polymorphism analysis of receptor gene of vitamin D gene (RVD) in venezuelan patients with osteoporosis.**  
*L. Borjas-Fajardo, M. Zambrano, E. Fernandez, L. Pineda, A. Morales-Machin, P. Romero, W. Zabala, AE. Rojas-Atencio, JA. Chacin, W. Delgado.* Unidad de Genetica Medica, Univ del Zulia, Maracaibo, Zulia, Venezuela.

Among genes implied on the study of the osteoporosis genetics, the most studied gene worldwide is the receptor gene of D vitamin (VDR), through the characterization of Bsm I polymorphism. This research had as a main objective to analyze the Bsm I polymorphism of the RVD gene in a sample of 133 postmenopausal women distributed in three groups: 54 with osteoporosis, 24 with osteopenia and 55 normal controls for the disease. PCR and conditions reactions were made like Morrison method. The results showed that 28 of the women with osteoporosis presented the BB genotype, which is related in others countries to DMO decrease, 20 had the Bb genotype, and 6 the bb genotype. Of the control group only 11 women presented the BB genotype, 36 showed the heterozygote genotype and 8 the bb genotype. The frequencies of the B and b alleles in the analyzed population were 0.6 and 0.4 respectively. The BB genotype was found in 52% of the group with osteoporosis, and in 20% of the control group, these findings are statistically significant, which suggest an association between the BB genotype and osteoporosis.

**Association of angiotensin-converting enzyme I/D polymorphism with vitiligo patients of Korea.** *S.Y. Jin<sup>1</sup>, H.H. Park<sup>2</sup>, M.H. Lee<sup>2</sup>, H.J. Lee<sup>1,3</sup>, M.S. Hong<sup>1,3</sup>, H.J. Park<sup>1</sup>, G.Z. Li<sup>3</sup>, J. Chung<sup>1,3</sup>.* 1) Department of Pharmacology, college of Medicine, Kyung Hee University, Seoul, Republic of Korea; 2) Department of Dermatology, college of Medicine, Kyung Hee University, seoul, Republic of Korea; 3) Kohwang Medical Research Institute, College of Medicine, Kyung Hee University, Seoul, Republic of Korea.

Vitiligo (leukoderma) is an acquired idiopathic hypomelanotic disorder characterized by circumscribed depigmented patches. It is a polygenic diseases, and the exact pathogenesis of vitiligo is not yet known. We report the association studies for the angiotensin-converting enzyme (ACE) gene I/D polymorphism in vitiligo patients. The ACE gene was selected as a candidate gene because of the ACE plays an important role in the physiology of vasculature, blood pressure and cutaneous inflammation and which has been widely investigated in its relation with various diseases including autoimmune diseases. ACE gene polymorphism in vitiligo patients has not been reported yet. In this study, ACE gene polymorphism is investigated in 120 Korean vitiligo patients and 429 healthy volunteers. The insertion (I) allele of the ACE gene polymorphism is associated with vitiligo susceptibility. The results ( $P < 0.05$ ) suggest an association of the mutation in ACE with the development of vitiligo in Korean patients.

**Analysis of SNPs reported to be associated with obesity.** *H. Lyon*<sup>1</sup>, *T. Bersaglieri*<sup>1</sup>, *K. Lunetta*<sup>2</sup>, *K. Ardlie*<sup>2</sup>, *J. Hirschhorn*<sup>1,3</sup>. 1) Genetics, Children's Hospital and Harvard Medical School, Boston, MA; 2) Genomics Collaborative Inc., Cambridge, MA; 3) Whitehead Institute Center for Genome Research, Cambridge, MA.

Obesity is now an epidemic in the United States. The increase in obesity will result in an increase in related diseases such as diabetes and heart disease. Understanding the genetic variation that contributes to obesity will be crucial for devising effective interventions. At least 71 single nucleotide polymorphisms (SNPs) in 55 genes have been associated with common obesity phenotypes, but these associations have not been consistently replicated (Chagnon et al 2002). Many of these associations are likely spurious, but some may represent true causal risk factors. To distinguish the true associations from the false leads, association analyses with large populations and well-defined phenotypes are necessary. We genotyped 22 of the SNPs previously reported to be associated with body mass index (BMI) using DNA samples supplied by Genomics Collaborative from a population of 1000 Polish people with BMI at the upper and lower ends of a population distribution. Obese subjects (n=700) had BMI from the 90th-97th percentile, while lean subjects (n=330) had BMI from 5th-12th percentile. A case-control chi square analysis using a SNP with 25% frequency had 97% power to detect a 1.5 fold odds ratio. The use of hypernormal controls further enhanced power to detect alleles with modest effects on BMI. None of the SNPs showed a significant association with obesity after correction for multiple tests. The 697G/C SNP in HT2RC (type 2C serotonin receptor) showed a nominal association of the minor allele (risk ratio for obesity=1.24, p value=0.04). Our study has thus far failed to replicate previously reported associations. This may be due to a high rate of false positive reports, differences between populations, testing alleles that are in linkage disequilibrium with, but not identical to the actual causal variants, or a lack of power for odds ratios below 1.25. To address these issues, we will assess the variants in a larger US Caucasian population of 2000 subjects, and use a comprehensive haplotype-based approach to assess the impact of genetic variation in these candidate genes.

**Analysis of DNA variants in the genes encoding the gastrin releasing peptide (GRP) and its receptor (GRPR) in panic disorder.** *S.P. Hamilton<sup>1</sup>, M. Durner<sup>2</sup>, L. Hodges<sup>1</sup>, M. Bautista<sup>1</sup>, O. Bravo<sup>1</sup>, G.A. Heiman<sup>2</sup>, T.G. Gilliam<sup>2</sup>, S.E. Hodge<sup>2</sup>, J.A. Knowles<sup>2</sup>, A.J. Fyer<sup>2</sup>, M.M. Weissman<sup>2</sup>.* 1) Dept. of Psychiatry, UCSF; 2) Depts. of Psychiatry and Genetics & Development, Columbia University College of Physicians and Surgeons.

Family, segregation, and twin studies imply a genetic component to the predisposition towards panic disorder. Few studies have investigated the potential involvement in panic disorder of genes encoding fear circuitry proteins. Recent animal studies suggest a role for the gastrin-releasing peptide (GRP) and its receptor (GRPR) in anxiety. Additionally, we observed single locus and multipoint heterogeneity lod scores of  $\sim 2.0$  approximately 15cM telomeric to the GRP gene in our recent published genome scan of a panic disorder syndrome. One consequence of the Human Genome Project has been the discovery of large numbers of DNA variations, including single nucleotide polymorphisms (SNPs), localized throughout the genome. The high density of these markers allows the construction of informative haplotypes for linkage disequilibrium mapping studies. Using high throughput methods for genotyping SNPs (FP-TDI and fluorogenic 5-nuclease), we have employed a family-based design to investigate the role of SNPs and simple tandem repeats in the genes for GRP and GRPR. We have genotyped  $\sim 1,200$  individuals in 120 panic disorder pedigrees, as well as 83 parent-proband triads. Subjects were genotyped at 1) 7 informative SNPs over 25kb in or around the GRP gene and 2 informative STRs flanking the GRP gene (18q21); and 2) 6 informative SNPs over 36kb at the GRPR locus (Xp22). These data are currently being analyzed for genetic association and linkage, and the results will be presented. In addition to standard linkage (using recessive/dominant genetic models, three diagnostic models, and tests of heterogeneity) and family-based association (FBAT) analyses, we plan to analyze the data using TRANSMIT, a program that compares the transmission of multilocus haplotypes from parents to affected offspring. Its main advantage is its ability to handle missing parental genotypes or phase-unknown parental genotypes.

**MTHFR C677T GENE MUTATION - A RISK MARKER FOR HEMIPLEGIA RELATED TO**

**VASCULOPATHIES AND COMPLICATIONS OF DIABETES.** *R.A. Varma<sup>1</sup>, S. Movva<sup>2</sup>, V. Mohan<sup>1</sup>, C.*

*Kamalesh<sup>1</sup>, J.R. Chaudary<sup>1</sup>, S. Saharia<sup>2</sup>, Q. Hasan<sup>1,2</sup>.* 1) Dept of Genetics, Kamineni Hospitals, Hyderabad, INDIA; 2) Bhagawan Mahaveer Hospitals and Research Center, Hyderabad, India.

**Introduction:** The enzyme methylenetetrahydrofolate reductase (MTHFR) is pivotal in the metabolism of homocysteine. A mutation (C677T) in the MTHFR gene results in a thermolabile enzyme with reduced activity. More and more studies indicate that polymorphisms in candidate genes, like MTHFR, increase the risk of diseases like hemiplegia, cardiovascular conditions and complications of diabetes. **Aim:** To evaluate the risk assessing potential of C677T mutation in the MTHFR gene in patients with hemiplegia or neurological problems related to vasculopathies and complications of diabetes. **Methods:** Patients problems related to vasculopathies (N=62) and diabetics with diabetic nephropathy and retinopathy (N=25) were included in the study. Controls included individuals having neurological conditions other than hemiplegia (N=15), diabetics of 10-year duration with no overt complications (N=10) and healthy age, sex-matched volunteers (N=35). Genomic DNA was isolated from heparinised blood using DNA isolation kit (Amersham Pharmacia). PCR was carried using specific primers for MTHFR. The amplified product was restriction digested with Hinf I (MBI Fermentas) to identify the mutation by agarose gel electrophoresis. **Results & Discussion:** The gene mutation was identified in 1/35 healthy controls, indicating that the prevalence of the mutation in this cohort is 2.85%. The MTHFR mutation was detected in 19/62 (30.64%) cases with neurological vasculopathies and the incidence of mutation was lower for neurological diseases other than hemiplegia and diabetics with nephropathy/retinopathy. **Conclusion:** There is a significant correlation between MTHFR C677T gene mutation and Neurological Vasculopathies. This can be considered as a risk marker in our population for hemiplegias. However, the sample size for the diabetic group both with and without complications may have to be increased prior to deciding whether the MTHFR gene mutation could be considered as a marker for these conditions.

**A novel approach to analyse genotype combinations in genes of the renin-angiotensin-aldosterone system in type 1 diabetic patients with and without diabetic nephropathy.** *K.J. Pettersson-Fernholm<sup>1</sup>, S. Sammalisto<sup>2</sup>, M.*

*Koivisto<sup>3</sup>, S. Frojdo<sup>1</sup>, M. Perola<sup>2</sup>, K. Sood<sup>3</sup>, M. Rinne<sup>1</sup>, C. Forsblom<sup>1</sup>, J. Fagerudd<sup>1</sup>, L. Peltonen<sup>2</sup>, PH. Groop<sup>1</sup>, FinnDiane Study Group.* 1) Folkhalsan Research Center, Biomedicum, Helsinki, Finland; 2) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 3) Department of Computer Science, University of Helsinki, Finland.

There is evidence for genetic involvement in the pathogenesis of diabetic nephropathy (DN) and the RAAS system, including the ACE, AT1, AT2 and CYP11B2 genes, is a possible candidate system. There are, however, no published data of combining more than 2 loci in DN. Therefore, the aim of this study was to study 9 polymorphisms in the RAAS system by using 3 approaches: 1) Conventional single locus analysis. 2) A novel computer program designed for multi-loci SNP analysis by using a pattern discovery framework called the naive Bayes model. The naive Bayes algorithm attempts to predict the phenotype from the given genotype combinations. 3) A multi-loci search computer program to test all possible 2- and 3-loci combinations. In a cross-sectional case-control study we studied 638 type 1 diabetic patients from 20 centers from the FinnDiane Study. Patients were classified based on their albumin excretion rate: NORMOalbuminuria (n=319) and PROTEinuria (n=319). The frequencies of rare genotypes of ACE I/D, CYP11B2 T-344C, 5 SNPs in the AT1 and 2 SNPs in the AT2 did not differ between the groups in the conventional analysis. However, in multi-loci analyses the naive Bayes model identified a significant deviation between the two groups. Further analyses showed that the following genotype combinations were more common in NORMO than PROT: CC of AT1 A1166C and CC of CYP11B2 T-344C (Fisher exact, p=0.004), ID of ACE I/D, CC of AT1 A1166C and CC of CYP11B2 T-344C (p=0.02) and AC of AT1 A+39C, ID of ACE I/D and CC of CYP11B2 T-344C (p=0.01). These preliminary results suggest that the naive Bayes method combined with a multi-loci search program is a powerful novel approach in the search for genotype combinations in DN and other complex traits.

**Population genetic approaches to Parkinsons disease.** *P.M. Abou-Sleiman<sup>1</sup>, D.G. Healy<sup>1</sup>, K. Ahmadi<sup>2</sup>, D.B. Goldstein<sup>2</sup>, N.W. Wood<sup>1</sup>.* 1) Department of Molecular Neuroscience, Institute of Neurology, Queen Square, London WC1N 3BG, United Kingdom; 2) Department of Biology, University College London, Darwin Building, Gower Street, London WC1E 6BT, United Kingdom.

Parkinsons disease is a neurological conditions of global health significance. A European population-based study estimated the age-dependant prevalence at 1.4, 2, and 3.4%; in those aged 55, 65 and 75 years or over, respectively. Over the past decade major advances have been made in elucidating rare Mendelian forms of the disease, however, very little is known about susceptibility factors influencing the common forms of the disease. We have used a multi-step programme based on linkage disequilibrium mapping to efficiently screen candidate genes for polymorphisms that predispose to PD. For this initial study we have selected 6 candidate genes, these are the two autosomal recessive genes parkin and DJ-1, three autosomal dominant genes -synuclein, NR4A2 and UCH-L1 and MAPT (tau), which has been reported to be associated with PD. We determined the haplotype structure of these candidate genes in a panel of European control trios by direct sequencing. We then determined a minimal set of SNPs that represent most of the observed haplotype diversity (haplotype tagging SNPs). The six genes combined, span a total of 1.7 Mb of genomic DNA, and we were able to capture an estimated 95% of the haplotype diversity within these genes using only 27 SNPs. The tagging SNPs were genotyped in 800 Parkinsons disease patients and a panel of 800 ethnically matched healthy controls using taqman allelic discrimination assays. We then used statistical approaches to identify haplotypes that predispose to PD in each individual gene. Additionally, we looked at possible interactions between the genes under investigation and the incidence of PD. Implicated gene regions were then re-sequenced to identify causal SNPs. These results will not only improve our understanding of this important neurological condition, but may also serve as a framework for the study of other common non-Mendelian diseases which have, to date, remained largely unresolved due to the inadequacy of the available methodologies.

**A range of known risk factors for conduct disorder interact with low MAO-A activity to increase risk. D.L.**

*Foley<sup>1</sup>, B.K. Wormley<sup>2</sup>, H.M. Maes<sup>1</sup>, J.L. Silberg<sup>1</sup>, L.J. Eaves<sup>1</sup>, B.P. Riley<sup>1, 2</sup>.* 1) Dept. of Human Genetics, Virginia Commonwealth Univ., Richmond, VA; 2) Dept. of Psychiatry, Virginia Commonwealth Univ., Richmond, VA.

Low MAO-A activity has been associated with antisocial behavior in boys exposed to maltreatment. We were interested to assess if other known risk factors for conduct disorder (CD) also interacted with low MAO-A activity to increase risk for CD. We therefore genotyped MAO-A polymorphisms in 516 boys from the Virginia Twin Study of Adolescent Behavioral Development and created a quantitative 6 item index of known risk factors for CD, which included parental neglect, exposure to inter-parental violence and inconsistent discipline. There was no main effect of MAO-A on risk for CD (OR= 0.80, CI=0.46, 1.39 P=0.43). Our index is composed of items known to predict CD and as expected we observe a significant main effect on risk for CD (OR=1.62, CI=1.20, 2.19, P=0.002). The bulk of this effect comes from the low activity group (OR=2.51 CI=1.46, 2.49, P=0.0008) although the high activity group is similar (OR=1.34, CI=0.94, 1.91, P=0.10). 27% of children had been exposed to 1+ index items. In an effort to identify those children with more severe risk factors for CD we set a threshold of 2+ index items (8% of the sample). In this more severely exposed group there was no main effect of index on risk for CD (OR=1.48, CI=0.79, 2.75 P=0.22) likely due to reduced power in this sub-sample. Critically, we do detect a statistically significant interaction between low MAO-A activity and the index regardless of the threshold used to define exposure. When exposure is defined by 1 or more index items the association between CD and the interaction between the index and low MAO-A activity is OR=1.87, CI=0.98, 3.56, P=0.03. When exposure is defined by 2 or more index items the association between CD and the interaction between the index and low MAO-A activity is even larger (OR=6.10, CI=1.54, 24.10, P=0.005). Our findings are therefore consistent with the results reported by Caspi et al from the Dunedin study, but they indicate that the low activity genotype interacts with a variety of risk factors for CD.

**Differential allele expression of MPDZ---A candidate gene for alcohol withdrawal liability.** *G. Zhu<sup>1, 2</sup>, P.J. Brooks<sup>1</sup>, D.C. Mash<sup>3</sup>, D. Goldman<sup>1</sup>.* 1) Laboratory of Neurogenetics, NIAAA, NIH, 12420 Parklawn Dr., Room 451, Rockville, MD 20852; 2) Changhai Hospital, Shanghai 200433, P.R. China; 3) Department of Neurology, University of Miami, Miami, FL 33136.

Previous studies show that risk for onset of alcoholism is associated with genetic differences in acute alcohol withdrawal severity. By using an animal model, in which DBA/2J(D2) and C57BL/6J(B6) mouse strains exhibit severe and mild acute alcohol withdrawal, respectively, a quantitative trait locus (QTL) for withdrawal was mapped to mouse chromosomes 4 (Buck et al. 1999). Congenic mapping narrowed the QTL to a 1.8 Mb interval and identified *Mpdz* (multiple PDZ domain protein) as a candidate gene (Fehr et al. 2002), but a causative coding sequence variant was not identified. We hypothesized that a quantitative difference in MPDZ expression might contribute to the difference in alcohol withdrawal. We used MPDZ coding sequence variants as endogenous reporters for MPDZ differential allele expression in brain tissues of both B6/D2 F1 mice and humans. RT-coupled 5 nuclease assays were developed for the SNP markers. Results: (1) In six B6/D2 F1 mouse brains, the B6-specific allele was consistently over-expressed relative to the D2-specific allele, with a mean allele ratio of 1.51 0.12. Real-time RT-PCR quantitation of *Mpdz* mRNA showed that B6 mice expressed 30% more *Mpdz* mRNA in brain than D2 mouse, which is consistent with the differential strain-specific allele expression. (2) In humans, differential MPDZ allele expression was found in 10/27 heterozygous postmortem brain samples, in which one allele is consistently higher expressed than the other allele, with a mean allele ratio of 1.60 0.23. These results indicate: (1) functional variants apparently exist in MPDZ regulatory region(s) in human, and these variants influence gene expression efficiency; (2) differential allele expression of MPDZ gene might be an important mechanism for alcohol withdrawal liability in human, as in mouse. Observation of conditional and dynamic change of *Mpdz* expression in acutely alcohol treated mice and linkage analyses between MPDZ genotype and/or haplotype and alcohol dependence in human are under way.

**Genetic Risk factors for Reduced Lung Diffusing Capacity In Middle Aged And Older Adults.** *M. Matheson<sup>1</sup>, J. Ellis<sup>2</sup>, J. Raven<sup>3</sup>, D. Johns<sup>4</sup>, EH. Walters<sup>4</sup>, M. Abramson<sup>1</sup>.* 1) Department of Epidemiology & Preventive Medicine, Monash University, Melbourne, Vic 3004; 2) Department of Physiology, The University of Melbourne, Vic 3010; 3) Department of Allergy & Respiratory Medicine, The Alfred, Vic 3004; 4) University of Tasmania, Hobart, Tas 7000.

**Introduction:** A reduced carbon monoxide diffusing capacity (DLco) and Kco (DLco/alveolar volume) in people with a significant smoking history is often associated with diseases of the lung parenchyma including emphysema. Emphysema may result from chemical and oxidative damage caused by reactive species present in cigarette smoke or released from neutrophils recruited following cigarette smoke induced injury. The glutathione S-transferase superfamily of detoxification enzymes may protect against such injury through detoxification of cigarette smoke components. The GSTM1 and GSTT1 genes are members of this family and contain polymorphisms that result in no enzyme production (null alleles). Our aim was to investigate the role of the GSTM1 and GSTT1 genes on DLco and Kco in subjects that were part of a cross-sectional study of Chronic Obstructive Pulmonary Disease in Melbourne Australia.

**Methods:** Participants in the study completed a detailed respiratory questionnaire, spirometry, and measurement of DLco and Kco by the single breath technique (n=1221). Polymorphisms in the GSTM1 and GSTT1 genes were determined from PCR analysis from previously published methods.

**Results:** Genotyping for GSTM1 and GSTT1 was completed for 1071 subjects. Individually neither the GSTM1 null nor the GSTT1 null genotypes were associated with reduced DLco or Kco. However inheritance of both the GSTM1 null and GSTT1 null genotype was associated with a significant reduction in diffusing capacity in smokers (p=0.036) allowing for age and gender.

**Conclusions:** These findings would suggest that GSTM1 and GSTT1 may play a role in the development of emphysema in those individuals exposed to cigarette smoke.

Program Nr: 2187 from 2003 ASHG Annual Meeting

**Transcriptional effects of the *EPM1* minisatellite.** L. Hsu, G. Zhang, T. Krontiris. Div Molecular Med, Shapiro Bldg, Beckman Res Inst City of Hope, Duarte, CA.

Expansion mutations of gene-associated minisatellites (VNTRs) are responsible for several human diseases, including myoclonus epilepsy (*EPM1*), insulin-dependent diabetes (*INS*), and neurodegenerative disorders (*5-HTT*, *MAOA*). To study the biological role of these repeat elements, we analyzed the effects of the *EPM1* VNTR on reporter gene activity in murine NIH3T3 fibroblasts and embryonic stem (ES) cells. In a transient transfection system, the expanded *EPM1* VNTR sequence (85 repeats) inhibited the reporter gene promoter activity both *in cis* and *in trans*, whereas the normal VNTR allele (3 repeats) did not alter promoter function. Downregulation of expression *in trans* strongly suggested the titration of certain transcription factors (the VNTR repeat unit bears an SP1 binding site), whereas expression inhibition *in cis* may additionally have resulted from the interaction of higher order VNTR structure with specific DNA-binding proteins. Expression downregulation by an expanded VNTR was also observed when the VNTR was stably incorporated into the 3T3 genome by transfection: Significantly fewer G418-resistant clones were obtained in NIH3T3 cells transfected with the insertion vector carrying an expanded *EPM1* VNTR. Four ES clones containing normal and expanded *EPM1* VNTRs (with 3, 20, 36, and 85 repeat units, respectively) attached to a reporter promoter region were obtained by employing a modified double-targeting strategy at the murine *mnk* locus. In contrast to differentiated 3T3 cells, the effect of VNTR length on *mnk* expression in targeted ES clones assayed by real time PCR was not significant. This result suggested that cell-type or developmental stage specific binding proteins may be involved in the transcriptional regulation by VNTR. We are now using several yeast and murine models to identify cellular DNA binding proteins that may mediate our observed VNTR effects.

**Examination of molecular characteristics of the *FMR1* gene: identifying risk factors for premature ovarian failure among female premutation carriers.** A.K. Sullivan, E.G. Allen, S.L. Sherman. Department of Human Genetics, Emory University School of Medicine, Atlanta, GA.

Premature ovarian failure (POF), defined as cessation of menses before age 40, occurs in 1% of the general population. *FMR1* female premutation carriers (PC) have a significantly increased risk for POF; however, the cause of their ovarian dysfunction is unknown. We hypothesize that molecular correlates related to the *FMR1* gene may explain this increased risk. Therefore, we examined *FMR1* CGG repeat size, X-inactivation ratio, *FMR1* transcript level, and parental origin of the *FMR1* premutation. We obtained reproductive histories on 403 women (271 with <59 repeats and 132 with 59 repeats) and FSH levels on a subset of these women (N=143). We confirmed previous findings that PC have an increased risk for ovarian dysfunction: they have a higher prevalence of POF (13%) and an earlier age at menopause (mean = 47.10.7). FSH level, an indicator of perimenopause, did not differ between PC and noncarriers under age 30. However, FSH levels were significantly higher in PC than in noncarriers among women in their 30s. Another indicator of ovarian dysfunction among PC was increased dizygotic twinning; however, the miscarriage rate was not increased significantly. Other reproductive measures did not differ between PC and noncarriers including age at menarche and variation in menstrual cycle characteristics. We conducted analyses to determine if repeat size, X-inactivation ratio and parental origin of the *FMR1* allele may explain the risk for POF among PC. We found that PC with 80 repeats had an earlier age at menopause and a higher prevalence of POF compared to those with smaller premutations. Parental origin was not found to be a risk factor for ovarian failure. X-inactivation ratio and transcript level were also not found to be risk factors, although the sample sizes were small for some of these analyses. These findings suggest that 1) PC may not have clinical problems associated with ovarian function in their earlier reproductive years and that 2) premutation size is a risk factor for ovarian dysfunction.

**Investigating Candidate Genes and Novel ESTs in Primary Open Angle Glaucoma.** *K.R. Abramson<sup>1</sup>, M.A. Hauser<sup>1</sup>, O.A. Marks<sup>1</sup>, C. Santiago<sup>1</sup>, F.L. Graham<sup>1</sup>, E.A. del Bono<sup>2</sup>, M.A. Pericak-Vance<sup>1</sup>, J.L. Wiggs<sup>2</sup>, J.L. Haines<sup>3</sup>, R.R. Allingham<sup>1</sup>.* 1) Duke University Medical Center, Durham, NC; 2) Massachusetts Eye and Ear Infirmary, Boston, MA; 3) Vanderbilt University, Nashville, TN.

We have previously reported linkage analysis in a set of 86 multiplex POAG families. (Wiggs et al, Hum. Mol. Genet., 9(7);1109-1117). Six genomic regions displayed linkage to POAG, including our strongest regions on 14q11 and 15q11-15q13. Phenotypic stratification using ordered subset analysis (OSA) has identified a subset of families with early onset disease (age at diagnosis less than 45 years) that are linked to the locus on chromosome 15. Candidate genes throughout the minimal candidate interval are being screened both by direct gene sequencing and by family-based association analysis. A sample set of 16 individuals representing 14 early onset families and two controls was used to sequence the known genes. Intron/exon boundaries were determined by BLAT comparison of the cDNA sequence and the UCSC genomic assembly. Each exon was pcr amplified and sequenced on an ABI3700, using standard protocols. The sequences were analyzed using PolyPhred and Sequencher software. Ten known genes in this region have been sequenced: ATP10C, GABRA5, GABRB3, GABRG3, APBA2, KIAA0574, TJP1, NDN, MAGEL2, and SNRPN. We have identified many SNPs, synonymous substitutions, and missense variants, but none of these variants appear to play a primary causative role in POAG. Association studies with 15 SNPs within the GABRA5, GABRB3, GABRG3 genes does not reveal any association with POAG disease status. We have also identified numerous ESTs located in this interval, including several that are expressed in ocular tissue. We present the genomic structure and mutation analysis of a number of ESTs in the 15q11.1-15q13.2 interval. ESTs were extended by 5'-RACE on BD Clontech Marathon-Ready retinal and fetal brain cDNA libraries. Subsequently these extended transcripts were chracterized and screened for mutations by sequencing.

**Evidence for digenic inheritance in adult-onset primary open angle glaucoma.** *M.K. Wirtz<sup>1</sup>, N.D. Gaudette<sup>1</sup>, J.R. Samples<sup>1</sup>, D. Choi<sup>1</sup>, G. Kitsos<sup>2</sup>, E. Economou-Petersen<sup>3</sup>, M. Grigoriadou<sup>4</sup>, G. Aperis<sup>4</sup>, P.L. Kramer<sup>5</sup>, K. Psilas<sup>2</sup>, M.B. Petersen<sup>4</sup>.* 1) Dept Ophthalmology, CE-Res, Casey Eye Inst/OHSU, Portland, OR; 2) Dept Ophthalmology, University of Ioannina, GREECE; 3) National Blood Derivative Center, Athens, GREECE; 4) Dept Genetics, Institute of Child Health, Athens, GREECE; 5) Dept Neurology, OHSU, Portland, OR.

Primary open angle glaucoma (POAG) is the most common form of glaucoma, a blinding disease that may affect as many as 105 million individuals worldwide according to the World Health Organization. Affected individuals have characteristic optic nerve damage with abnormal visual fields, increased optic cup to disc ratios ( $>0.7$ ) and often high intraocular pressures ( $>21$  mm Hg). We have previously mapped a gene for POAG to chromosome 3, *GLC1C*, in two large families. To determine if more than one gene for POAG might be segregating in these families, we screened family members for mutations in *MYOC*, the *GLC1A* gene. *MYOC* variants in the US *GLC1C* family from Oregon included the Y347Y polymorphism in several family members and K398R in a spouse. In the Greek *GLC1C* family from Epirus, *MYOC* base pair changes included T377M, D380H in a spouse, and the Y347Y variant. The T377M change was found in 7 out of 10 affected POAG members. All affected family members carried the *GLC1C* haplotype. Four family members had the T377M *MYOC* variant and a normal haplotype at the *GLC1C* locus. None of these individuals have POAG at this time; all of them appear to have normal cup to disc ratios and only one shows evidence of elevated intraocular pressure. However, the oldest is only 50, thus, it will be important to monitor these individuals over time. The T377M mutation has been identified in Australian families with Greek or Macedonian ancestry. Screening 60 random POAG s Greek patients identified one individual with the T377M variant and four with the Y347Y polymorphism. Haplotype studies are in progress to determine if the T377M variant results from a single founder in the Greek population. This is the first report of potential digenic inheritance in the more common late-onset form of high pressure primary open angle glaucoma.

Program Nr: 2191 from 2003 ASHG Annual Meeting

**Exclusion of TGF Beta-Induced Factor as a Candidate Gene for MYP2 Autosomal Dominant High Myopia.** *T. Young, G. Scavello, P. Paluru, W. Ganther.* Divisions of Ophthalmology and Genetics, Children's Hosp of Philadelphia, Philadelphia, PA.

**PURPOSE:** TGF Beta-Induced Factor (TGIF) is a TALE homeobox gene that maps within the MYP2 high myopia locus interval at chromosome 18p11.31. In a recent report, TGIF was implicated as the MYP2 gene by SNP association studies of exon 3 in 1 transcript variant. (1) We systematically screened for nucleotide variations in the entire TGIF gene, examining an additional 7 transcript variants.

**METHODS:** TGIF was screened for sequence variants in the 7 original MYP2 families by direct DNA sequencing. The TGIF model used was the 4/20/03 human genome NCBI build #33, which has 10 exons and encodes for 8 transcript variants. Polymorphic sequence changes were compared to the previous report.

**RESULTS:** We observed 6 polymorphisms in 8 transcript variants of 10 exons, 3 of which were missense mutations and 3 were silent. The 3 missense variances localized to exon 10 at positions 235CT(ProLeu), 243CT(ProSer), and 244CT(ProLeu). Silent mutations were observed on exon 10 at positions 187AG, 332CT, and 413TG. Five polymorphisms were novel. Of the 25 SNPS published in the earlier report, only one - 187AG (804AG as previously noted) - was observed in our investigation. No sequence alterations co-segregated with the affected disease phenotype.

**CONCLUSIONS:** Mutation analysis of the full TGIF gene for MYP2 autosomal dominant high myopia did not identify sequence alterations associated with the disease phenotype. Further studies of MYP2 candidate genes are needed to determine the gene causative for this potentially blinding disorder.

1. Lam DS, et al. Invest Ophthalmol Vis Sci. 2003;44:1012-15.

Acknowledgements: NIH, Research To Prevent Blindness, Inc., Mabel E. Leslie Research Funds.

**A novel missense mutation within the DNA-binding domain of GCMB causes autosomal recessive isolated hypoparathyroidism.** L. Baumber<sup>1</sup>, S. Patel<sup>1</sup>, C.A. Johnson<sup>2</sup>, E.R. Maher<sup>2</sup>, R.C. Trembath<sup>1</sup>. 1) Department of Genetics, University of Leicester, Leicester, UK; 2) Section of Medical & Molecular Genetics, University of Birmingham, Birmingham, UK.

Familial isolated hypoparathyroidism refers to an uncommon metabolic disorder characterized by hypocalcemia and hyperphosphatemia, due either to abnormal activity or deficiency of parathyroid hormone (PTH). We recently identified a consanguineous Pakistani family with multiple affected individuals, each with isolated hypoparathyroidism presenting with seizures soon after birth. Disease segregation was compatible with an autosomal recessive mode of inheritance, so we initially used autozygosity mapping to exclude linkage to the PTH locus in this family. Subsequent microsatellite marker analysis identified a region of homozygosity flanking the *GCMB* (glial cells missing, *Drosophila* homologue B) gene. GCMB belongs to a family of transcription factors containing a novel N-terminal DNA-binding domain. This gcm motif is highly conserved in *Drosophila* and mouse homologues, with human isoforms A and B displaying additional homology to their mouse counterparts outside this region. Mouse GCMB, expressed predominantly in parathyroid tissue, is critical for parathyroid gland development in mice. A deletion within *GCMB* has previously been shown to cause hypoparathyroidism in a single family. Here we report a novel missense mutation within the DNA-binding domain of this gene. Sequencing all five exons of the *GCMB* gene, we have identified a homozygous R47L substitution, present in all affected individuals in this family. Unaffected parents were heterozygous for the mutation, which was not detected in any of 108 chromosomes screened in a control cohort of unrelated Pakistani individuals. This study provides further evidence of a critical role for GCMB expression and activity in parathyroid development in humans. The identification of this novel mutation, the first amino-acid substitution reported within the conserved DNA-binding motif of GCMB, suggests hypoparathyroidism in this family is caused by altered transcriptional regulation of downstream genes, a hypothesis now amenable to direct experimental examination.

**Functional interaction between BMPR2 and Tctex-1, a light chain of Dynein, is isoform-specific and disrupted by mutations underlying primary pulmonary hypertension.** *R.D. Machado*<sup>1</sup>, *N. Rudarakanchana*<sup>2</sup>, *N. Morrell*<sup>2</sup>, *R. Trembath*<sup>1</sup>. 1) Division of Medical Genetics, University of Leicester UK; 2) Department of Medicine, University of Cambridge UK.

Primary pulmonary hypertension (PPH) is characterised by the obstruction of pulmonary arterioles leading to a sustained elevation of pulmonary arterial pressure. PPH is usually fatal, typically as a result of right heart failure. Heterozygous mutations in the bone morphogenetic protein receptor type II (*BMPR2*) gene, a receptor of the TGF family, have been shown to cause PPH. Two isoforms of *BMPR2*, distinguished by the presence of a long cytoplasmic tail encoded by exon 12 of the gene, transduce signal by phosphorylating cytoplasmic mediators upon forming heteromeric complexes with type I receptors. A third of PPH mutations occur in the long C-terminal cytoplasmic tail, a domain of unknown function and unique to *BMPR2*. As the generation of the short isoform, lacking exon 12, is unlikely to be affected by these mutations, we investigated the cellular function of the cytoplasmic domain. Using a yeast two-hybrid screen and *in-vitro* binding assays we have identified Tctex-1, a light chain of the multi-subunit cytoplasmic dynein microtubule motor, as a binding partner to the *BMPR2* cytoplasmic tail. Dynein is required for the intracellular trafficking of protein cargo, the specificity for which depends on the composition and phosphorylation of sub-units in the complex. We demonstrate that the binding affinity of the short isoform of *BMPR2* for Tctex-1 is much lower than that of the long variant. Through kinase assays, we have shown that Tctex-1 is phosphorylated by *BMPR2*. Predictably, kinase mutation removed the ability of *BMPR2* to phosphorylate Tctex-1. Cytoplasmic tail *BMPR2* mutant constructs, both truncating and missense, were able to bind Tctex-1 at similar levels to the long isoform but unable to phosphorylate it. These data emphasise the requirement of the cytoplasmic domain of *BMPR2* in mediating functional activity. As interaction with Tctex-1 is disrupted by PPH causing mutations in *BMPR2*, further investigation of the role of the light chain in the regulation of *BMPR2* signalling is now required.

Program Nr: 2194 from 2003 ASHG Annual Meeting

**Pendrin Syndrome - A Complex Disease.** *T. Yang*<sup>1</sup>, *K. Kolln*<sup>1</sup>, *S. Enerback*<sup>2</sup>, *R. Smith*<sup>1</sup>. 1) Department of Otolaryngology and Interdepartmental PhD Program in Genetics, University of Iowa, Iowa City, IA 52242, USA; 2) Medical Genetics, Department of Medical Biochemistry, Goteborg University, Goteborg, Sweden.

Pendred syndrome (PDS) is the most common form of syndromic deafness. It is an autosomal recessive disorder characterized by severe-to-profound sensorineural hearing loss (SNHL), thyroid goiter and temporal bone malformations. Mutations in SLC26A4 are the major genetic cause of PDS, contributing approximately 50% to the PDS genetic load. An additional 10% of persons with PDS segregate mutations in FOXI1. This gene is an important regulator of SLC26A4 expression in the inner ear. Based on murine experiments, Foxi1 acts upstream of Slc26a4; homozygous mouse mutant knockouts of either gene show identical phenotypes (Hulander et al., 2003). In many families segregating a PDS phenotype, only a single disease-causing mutation is found in either FOXI1 or SLC26A4. This finding suggests that other genes modify the PDS phenotype. We hypothesize that many cases of PDS represent double heterozygosity. To test this hypothesis, we are screening the promoter region of SLC26A4 for mutations that may affect binding of FOXI1. In addition, we are completing a yeast-two-hybrid study to identify interacting partners of Foxi1, using as preys E9 and P9 cochlear libraries. This research was supported by R01-DC02842 (RJHS).

**DFNB1-Causing Mutations Outside the Coding Region of GJB2.** *H. Azaiez<sup>1</sup>, R. Tom Taggart<sup>2</sup>, I. Del Castillo<sup>3</sup>, S. Prasad<sup>1</sup>, C. Nishimura<sup>1</sup>, G. Van Camp<sup>4</sup>, R.J.H. Smith<sup>1</sup>.* 1) Dept Otolaryngology, Univ Iowa, Iowa City, IA, USA; 2) Dept of Pediatrics, School of Medicine, SUNY at Buffalo, NY, USA; 3) Unidad de Genetica Molecular, Hospital Ramon y Cajal, Madrid, Spain; 4) Dept of Medical Genetics, University of Antwerp, Antwerp, Belgium.

Mutations in GJB2, encoding the protein Connexin 26 (Cx26), account for half of recessive deafness in many different populations, making deafness at the DFNB1 locus one of the worlds most common genetic conditions. Frequently, only a single deafness-causing allele variant of the coding region of GJB2 is identified. This finding is not uncommon and implies the presence of a missed mutation outside the coding region of GJB2 or coincidental carrier status in a person with deafness of another etiology. To address this question, we screened 1088 deaf persons lacking two non-complementary mutations of GJB2. Of these persons, 100 carried a single GJB2 allele variant of exon 2; 988 persons carried two wild-type alleles. Within the group of 100 carriers, we found compound heterozygosity for IVS1+1G-A (n=5) and (GJB6-D13S1830) (n=7), the two most frequently reported DFNB1 mutations outside the coding region of GJB2. Of the remaining 88 deaf persons in whom a second GJB2 deafness-causing mutation could not be found, 37 were 35delG heterozygotes. Based on a 35delG carrier rate of 2.5%, in a cohort of 1076 deaf persons we would anticipate identifying 27 deaf 35delG carriers. This discrepancy suggests the existence of at least one additional DFNB1-causing mutation outside the coding region of GJB2. To screen for this putative mutation, we sequenced GJB6, the promoter and intron of GJB2, and select genomic regions conserved between human and mouse. No obvious disease-causing mutations were identified. We then typed several SNPs uniformly distributed throughout the interval bounded by GJB2 and CRYL1 in these patients and in 35delG homozygotes and normal-hearing controls. Comparison of the SNP distribution in these three populations suggests the existence of at least one additional DFNB1-causing mutation outside the coding region of GJB2. This research was supported by R01-DC02842 (RJHS).

**Relative frequencies of the mitochondrial A1555G and 961 del T mutations in the 12SrRNA gene in a large sample of deaf probands from the United States.** *K. Arnos<sup>1</sup>, X.J. Xia<sup>2</sup>, G. Norris<sup>1</sup>, B. Landa<sup>2</sup>, R. Falk<sup>3</sup>, R. Erdenetungalag<sup>4</sup>, B. Dangasuren<sup>4</sup>, W.E. Nance<sup>2</sup>, A. Pandya<sup>2</sup>.* 1) Dept Biology, Gallaudet Univ, Washington, DC; 2) Dept of Human Genetics, Virginia Commonwealth Univ, Richmond, VA; 3) Dept of Medical Genetics, Cedar Sinai Med Ctr. Los Angeles; 4) Maternal & Child Health Research Ctr,Ulaanbataar, Mongolia.

Mitochondrial mutations are being recognized to be of growing importance as a cause for deafness. The A1555G mutation in the 12SrRNA gene is a well established cause of deafness due to aminoglycoside ototoxicity. Its prevalence varies in different ethnic groups, with high frequencies among deaf from East Asia and Spain, and a lower frequency in the US. Since some individuals with the A1555G mutation do not develop hearing loss (HL) while others develop HL without exposure to aminoglycosides, additional genetic modifiers have been suggested to alter the phenotype. Casano et al.(1999) reported a second mutation in the 12SrRNA gene, the deletion of a thymidine at bp 961 also associated with aminoglycoside ototoxicity, independent of A1555G mutation. Others have reported the co-occurrence of both mutations in deaf probands from China (Bacino 1995, Li 2002). Screening of 1173 anonymized newborn blood spots in Texas yielded incidences of 0.08 and 0.59 for the A1555G and 961 del T mutations respectively (Tang, 2002). The hearing status of these newborns, however, is not known. We have determined the frequency of these variants in 721 DNA samples in a national repository of deaf probands from the US along with samples from 713 deaf probands from Mongolia. We did not identify a single proband with the A1555G mutation but the 961 del T mutation occurred with a frequency of 1.2% in the US. A matrilineal transmission was evident in one of the families. A definite history of aminoglycoside use was not present in most. In contrast, the incidence of 961 del T in Mongolia was 2.8%, and 16 of these subjects also carried the A1555G mutation. These data raise questions about the independent contribution of the 961 del T mutation to HL. A prospective study on newborns with both physiologic and molecular screen for HL, may help resolve some of these questions.

**Second DFNA11 mutation found in *MYO7A* gene.** *M.W.J. Lijndijk*<sup>1</sup>, *E. van Wijk*<sup>2</sup>, *E. Krieger*<sup>3</sup>, *A.M.L.C. Bischoff*<sup>2</sup>, *C.W.R.J. Cremers*<sup>2</sup>, *F.P.M. Cremers*<sup>1</sup>, *H. Kremer*<sup>2</sup>. 1) Human Genetics, Univ Med Cntr Nijmegen, Nijmegen, Netherlands; 2) Otorhinolaryngology, Univ Med Cntr Nijmegen, Nijmegen, Netherlands; 3) Center for Molecular and Biomolecular Informatics, Univ Med Cntr, Nijmegen, Netherlands.

Myosin IIVA is an unconventional myosin that has been implicated in Usher syndrome type 1B, in non-syndromic recessively inherited (DFNB2) and dominantly inherited (DFNA11) hearing impairment. Unconventional myosins are actin-based motor molecules that transduce chemical energy derived from ATP into a force enabling them to move along actin filaments.

A family presenting with autosomal dominant bilateral hearing loss most progressive in the higher frequencies was studied. The clinical data suggested DFNA11 to be the type of hearing loss inherited in this family. Linkage analysis confirmed this and mutation analysis of the *MYO7A* gene was performed.

When compared to the published sequence 11 changes were found. Seven polymorphisms were found in the protein coding sequence of the gene and three polymorphisms were intronic changes that were predicted not to cause significantly altered splicing. A c.1373 AT transversion that is heterozygously present in all affected family members, co segregates with the disease and was not found in 300 control individuals. The nucleotide change results in the amino acid substitution N458I.

N458 is located in a region of the myosin VIIA motor domain that is highly conserved throughout species and myosins. To estimate the likelihood that the observed N458I mutation is indeed responsible for the patients symptoms, a molecular model of myosin VIIA was built based on the known structure of the myosin II heavy chain from *Dictyostelium discoideum*. In the model, N458 is a conserved residue that is part of the switch II -helix and fulfills an important structural role by attaching the helix to the preceding switch II loop. In the model, the N458I mutation has severe effects.

**A novel connexin 26 (GJB2) mutation in a case of sensorineural hearing loss, keratoderma and knuckle pads.** *N. Leonard<sup>1</sup>, A.L. Krol<sup>2</sup>, S.M. Hasse<sup>1</sup>, K. Sprysak<sup>1</sup>, M.J. Somerville<sup>1</sup>*. 1) Dept Medical Genetics, Univ Alberta, Edmonton, AB, Canada; 2) Oregon Health and Science University, Portland, OR, USA.

We report on an eight-year-old girl who developed striate linear lesions of keratoderma on the palms and knuckle pads on the dorsum of her fingers. Striate linear lesions were developing on the soles. She had mild hyperkeratosis over one elbow. She showed no evidence of restricting lesions or diffuse keratoderma as seen in Vohwinkel syndrome (MIM 124500). Her teeth, hair and nails were normal with no evidence of leukonychia. She was diagnosed with a non-progressive congenital mild sloping to severe sensorineural hearing loss bilaterally. She otherwise was healthy. There are no other individuals in family history with hearing loss or dermatologic changes. In particular her parents and sister were unaffected. Sequencing of the connexin 26 (GJB2) gene showed a single mutation G59R (175G>C) in exon 2. This mutation had not been described before, however a similar mutation within the same codon (G59A) had previously been reported to cause an autosomal dominant syndrome of hearing loss and palmoplantar hyperkeratosis without knuckle pads (Heathcote K et al., J Med Genet 2000;37:50-51). The G59R mutation seen in our case was not detected in either parent. Paternity was confirmed by a genome-wide multiplex analysis of 15 tetranucleotide repeat loci. Our patient shows overlap of clinical features with palmoplantar keratoderma with deafness (MIM 148350). She does not show severe features as seen in Vohwinkel (Deafness, congenital, with keratopachydermia and constrictions of fingers and toes - MIM 124500). Both of these conditions have shown connexin 26 mutations. Bart-Pumphrey (Knuckle Pads, leukonychia, and sensorineural deafness - MIM 149200) shares features of deafness and knuckle pads similar to our patient. She however did not have leukonychia. To date a gene mutation has not been described for this condition or for conditions involving knuckle pads. The connexin 26 mutation in our patient suggests there may be overlap amongst the keratodermas, stemming from different mutations in the connexin 26 (GJB2) gene.

**GJB2 mutations in Indian families with Autosomal Recessive Nonsyndromic Sensorineural Hearing Loss (ARNSHL).** *R. Vijaya, M. Kabra, P.S.N. Menon, R.C. Deka\**. Genetics Unit, Department of Pediatrics and \*Department of Otorhinolaryngology, All India Inst Medical Sci, New Delhi, India.

Mutations in the gene GJB2 are responsible for more than 50% cases of prelingual nonsyndromic deafness in Caucasian populations. The aim of the study was to characterize the GJB2 mutations in Indian families with Autosomal Recessive Nonsyndromic Sensorineural Hearing Loss (ARNSHL). Totally 437 deaf individuals belonging to 202 families with ARNSHL (123 multiplex families and 79 simplex families) were screened for mutations in the coding region of the GJB2 gene and 211 ethnically matched controls were screened to estimate the carrier frequency of the various mutant alleles. GJB2 mutations account for 28.8% of the mutant alleles in the multiplex families, 12% of the mutant alleles in the simplex families and 25.7% of all GJB2 deafness alleles studied. Six previously reported mutations W24X, W77X, Q124X, del313-326, 35delG & V37I and one novel mutation E147X were identified. In addition, three polymorphisms R127H, V153I and R165W were also detected. The most common mutation was W24X contributing to 87.55% of the GJB2 mutated alleles and was found to be a major cause for nonsyndromic deafness in Indian families with a carrier frequency of 1.4% in general population. The high prevalence of W24X indicates a founder effect rather than a mutational hot spot in the Indian population. The most common mutation in Caucasian population, the 35delG, occurs as a rare allele but the other common mutations 167delT, R143W and 235delC were not found. All individuals with GJB2 mutations had significant prelingual, usually bilateral, sensorineural, non-progressive and severe to profound hearing loss. The present study reveals that approximately 25% of the ARNSHL families including the simplex cases are due to mutations in the GJB2 gene and indicate that mutations in GJB2 gene are an important contribution to nonsyndromic recessive deafness in Indian ethnic groups. Though the other mutations are less frequent, the high prevalence of W24X helps in evolving a strategy to screen for the mutations in the GJB2 gene in patients with ARNSHL as part of the genetic testing.

**Heterozygous mutation in promoter region of SPR results in Dopa-responsive dystonia.** *U. Muller<sup>1</sup>, N. Blau<sup>2</sup>, D. Goriounov<sup>2</sup>, J. Bitsch<sup>1</sup>, S. Hummel<sup>3</sup>, D. Steinberger<sup>1,4</sup>.* 1) Inst Human Genetics, Justus-Liebig Univ, Giessen, Germany; 2) Division of Clinical Chemistry and Biochemistry, University Childrens Hospital, Zurich, Switzerland; 3) Neurologische Unisversitatsklinik Freiburg, Freiburg, Germany; 4) Center for Human Genetics Ingelheim, Ingelheim, Germany.

In about 50 % of patients with Dopa-responsive dystonia (DRD) no mutations in the GCH1 gene can be detected. GCH1 codes for GTP-cyclohydrolase I, the rate-limiting enzyme in the biosynthesis of tetrahydrobiopterin. We argued that at least some of the GCH1-negative cases of DRD are caused by mutations in other genes involved in the biosynthesis of biopterin. One of these genes, SPR, codes for sepiapterin reductase (SR [E.C.1.1.1.153]) that converts 6-pyruvoyl-tetrahydropterin (PTP) to tetrahydrobiopterin (BH4). We tested for mutations in SPR in 95 GCH1-negative DRD cases and found the heterozygous base exchange ga at position 13 of the promoter region of one patient, a 26 year-old woman. She had developed fixed pes equinovarus during childhood. The symptoms disappeared before puberty and at age 15 she noticed abnormal movements of 4th and 5th digits of the left hand, at age 19 she developed gait anomalies with internal rotation, adduction, and extension of the left leg. Presently, the major findings are gait anomalies primarily caused by dystonia of left leg, tremor of the left arm, dystonia of digits 4 and 5 of the left hand, and involvement of the vocal cords. There was a positive response of signs and symptoms to L-dopa (3x200mg Madopar/die). Analysis of SR in patients fibroblasts showed significant reduction in activity consistent with a pronounced negative effect of the SPR mutation on SR production.

Program Nr: 2201 from 2003 ASHG Annual Meeting

**Region specific changes of gene expression in a transgenic mouse model for Huntington disease.** *F. Oyama, H. Miyazaki, N. Sakamoto, N.R. Jana, S.E. Kotliarova, N. Nukina.* Lab CAG Repeat Dis/Molec Neuro, RIKEN Brain Science Inst, Saitama, Japan.

Huntington disease is a hereditary neurodegenerative disorder caused by expansion of the CAG repeat in the exon 1 of the huntingtin (htt) gene. We established transgenic line expressing truncated form of human Nhtt (tNhtt) with 180 poly Q tract fused with EGFP (HD180) and analysed altered gene expressions by GeneChip technology profiling from brain of HD180. We found that a large number of gene expressions in the brain are changed in HD180 comparing with their control littermates. Majority of differentially expressed genes were down-regulated. We further confirmed the altered gene expression especially related to the nervous system function by RT-PCR assay, followed by in situ hybridization and found that vasopressin, oxytocin, enkephalin, prodynorphin, substance P mRNAs and several ESTs are down-regulated in the HD180. The results suggest that these changes are due to the region-specific degeneration and dysfunction of hypothalamic nuclei as well as striatum in the brains of HD180.

**Tyrosinase gene mutations in oculocutaneous albinism 1 (OCA1): definition of the phenotype.** *R. King<sup>1</sup>, J. Pietsch<sup>1</sup>, J.P. Fryer<sup>1</sup>, S. Savage<sup>1</sup>, M.J. Brott<sup>1</sup>, I. Russell-Eggitt<sup>2</sup>, C.G. Summers<sup>3</sup>, W.S. Oetting<sup>1</sup>.* 1) Medicine/Institute of Human Genetics Univ Minnesota, Minneapolis, MN; 2) Great Ormond Street Hospital for Children, London, UK; 3) Ophthalmology, Univ Minnesota, Minneapolis, MN.

Oculocutaneous albinism (OCA) is a common human genetic disease resulting from mutations in at least ten different genes. OCA1 results from mutations of the tyrosinase gene and presents with the life-long absence of melanin pigment after birth (OCA1A) or with the development of minimal-to-moderate amounts of cutaneous and ocular pigment (OCA1B). Other types of OCA have variable amounts of cutaneous and ocular pigment. We hypothesized that white hair at birth indicates OCA1 and tested this hypothesis in a sample of 120 probands with OCA who had white hair at birth. We found that 102(85%) of the probands had OCA1. Most probands were compound heterozygotes, and 169 of the 204 OCA1 tyrosinase gene alleles had identifiable mutations, while 35(17%) had cryptic or hidden mutations that were not identified with standard sequencing techniques that analyzed the coding, splice junction or proximal promoter regions of the gene. The cryptic mutations were more common in OCA1B (24/35,69%) than in OCA1A (11/35,31%). Tyrosinase gene mutations were distributed across exons 1-4 with few in exon 5. Seven probands with no tyrosinase gene mutations were found to have OCA2 with one or two P gene mutations, and eleven probands had no mutations in either the tyrosinase or the P genes. We conclude that the presence of white hair at birth is a useful clinical tool that suggests OCA1 in the initial evaluation of a child or adult with OCA, but that some probands with OCA2 may also have this presentation, indicating that molecular analysis of the tyrosinase and P genes are necessary for accurate diagnosis. Furthermore, the presence of cryptic mutations of the tyrosinase gene, particularly in OCA1B, suggests that more complex mutation mechanisms of this gene exist in OCA.

**Loss-of-function of an *N*-acetylglucosaminyltransferase, POMGnT1, in muscle-eye-brain disease.** *K. Kobayashi*<sup>1</sup>, *H. Manyá*<sup>2</sup>, *T. Toda*<sup>1</sup>, *T. Endo*<sup>2</sup>. 1) Div Functional Genomics, Dept Post-Genomics and Diseases, Osaka Univ Grad Sch Med, Osaka, Japan; 2) Glycobiol Res Gr, Tokyo Metropolitan Inst of Gerontol, Tokyo, Japan.

Muscle-eye-brain disease (MEB) is an autosomal recessive disorder characterized by congenital muscular dystrophy, ocular abnormalities, and lissencephaly. Mammalian *O*-mannosyl glycosylation is a rare type of protein modification that is observed in a limited number of glycoproteins of brain, nerve, and skeletal muscle. We isolated a human cDNA for protein *O*-mannose 1,2-*N*-acetylglucosaminyltransferase 1 (POMGnT1). POMGnT1 participates in the synthesis of *O*-mannosyl glycan, a modification that is known to be a laminin-binding ligand of -dystroglycan. Although 13 disease-causing mutations of the *POMGnT1* gene have been identified in patients with MEB, only the protein with the most frequently observed splicing site mutation has been studied previously. This protein was found to have no activity. In the present study, we expressed the remaining mutant POMGnT1 and found all mutants lost activity entirely. The results clearly demonstrate that MEB is inherited as a loss-of-function of POMGnT1. Since the MEB phenotype overlaps substantially with those of Fukuyama-type congenital muscular dystrophy (FCMD) and Walker-Warburg syndrome (WWS), these three diseases are thought to result from a similar pathomechanism, post-translational disruption of dystroglycan-ligand interactions. Our findings would propose a novel pathomechanism, glycosylation, on muscular dystrophy as well as neuronal migration disorder. Future studies will expand this new glycopathology field.

**Male infertility due to germ cell apoptosis in mice lacking the thiamin carrier, *Tht1*: a new insight into the critical role of thiamin in spermatogenesis.** *K. Oishi*<sup>1</sup>, *M. Barchi*<sup>2</sup>, *B.D. Gelb*<sup>1</sup>, *G.A. Diaz*<sup>1</sup>. 1) Department of Human Genetics, Mount Sinai School of Medicine, New York, NY; 2) Cell Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY.

Thiamin-responsive megaloblastic anemia (TRMA) is an autosomal recessive syndrome with megaloblastic anemia, sensorineural deafness, and diabetes mellitus. The disease gene, *SLC19A2*, encodes the high affinity thiamin carrier, THT1. A mouse model of TRMA lacking functional *Slc19a2* has been generated and showed a similar phenotype when maintained on a thiamin-free diet. In mice maintained on standard chow, an unexpected male-specific sterility phenotype was observed, while females reproduced normally. We characterized the testis-specific effects of absence of the high-affinity thiamin transporter, Tht-1. Null males were infertile due to aspermia and found to have hypoplastic testes (*Slc19a2*<sup>-/-</sup>: 36.31.5 vs. *Slc19a2*<sup>+/+</sup>: 72.54.0 mg;  $P < 0.00001$ ). Morphologic and testis-specific gene expression analyses revealed that spermatocyte development was completely arrested at the pachytene stage under conditions of standard thiamin intake, while tissues affected in the syndrome (pancreatic  $\beta$ -cell, hematopoietic cells, auditory nerve) maintained normal function. Using TUNEL and phospho-histone-H3 staining assays, we confirmed the stage of spermatogenesis arrest and determined that the lack of Tht1 in testis caused germ cell apoptosis. After thiamin deficiency challenge for 16 days, the apoptotic cell loss extended to earlier stages of germ cells but spared Sertoli and Leydig cells. Intraperitoneal injection of high-dose thiamin (1 mg/day for 20 days) was effective in reversing the spermatogenesis failure and mature sperm were produced, suggesting that the absence of the thiamin carrier could be overcome by diffusion-mediated transport at supranormal thiamin concentrations. These observations demonstrated that male germ cells, particularly those beyond the blood-testis barrier, are more susceptible to apoptosis triggered by intracellular thiamin deficiency than any other tissue type. The findings highlight an unexpected and critical role for thiamin transport and metabolism in spermatogenesis.

**Exclusion of candidate genes in four families with autosomal dominant multiple epiphyseal dysplasia.** *M. Czarny-Ratajczak*<sup>1,3</sup>, *P. Rogala*<sup>4</sup>, *A. Latos-Bielenska*<sup>3</sup>, *L. Ala-Kokko*<sup>1,2,5</sup>. 1) Center for Gene Therapy, Tulane University, New Orleans, LA; 2) Department of Medicine, Tulane University, New Orleans, LA; 3) Department of Medical Genetics, Karol Marcinkowski University of Medical Sciences, Poznan, Poland; 4) Department of Orthopedics, Karol Marcinkowski University of Medical Sciences, Poznan, Poland; 5) Collagen Research Unit, Biocenter and Department of Medical Biochemistry and Molecular Biology, University of Oulu, Finland.

Multiple epiphyseal dysplasia (MED) is a disorder of the skeletal system limited to cartilage and manifested as disturbance in the development of the epiphyses. The first symptoms of MED occur in childhood and include joint pain, waddling gait as well as restriction of joint mobility. Thus far mutations in five genes, COL9A1, COL9A2, COL9A3, COMP and MATN3 have been identified in MED. However, mutations in these five genes are only found in about half of the patients. We studied here four families with autosomal dominant MED. The families had six to fourteen affected members. A detailed clinical and radiological examination was performed on all the probands and affected family members. Four intragenic markers and nine microsatellite markers were used for linkage analysis of the COL9A1, COL9A2, COL9A3, COMP, MATN3 genes in four families. Linkage analysis of markers showed recombination between the five candidate genes and the phenotype in three families. Recombinations were observed between the COL9A1, COL9A2 and COMP genes in the fourth family. The proband of the fourth family was analyzed for mutations in the COL9A3 and MATN3 genes. Mutation analysis consisted of sequencing of the candidate exons, exon 2 of the MATN3 gene and exons 2 to 4 of the COL9A3 gene. The analysis failed to identify mutations. The results suggest the presence of additional locus/loci for MED. We are currently performing genome-wide screening in these families to identify new loci for MED.

**Novel mutations in EVC and EVC2 in Ellis-van Creveld Syndrome.** *M. Galdzicka<sup>1</sup>, G.E. Graham<sup>2</sup>, J.F. Cai<sup>1</sup>, S. Patnala<sup>1</sup>, M.G. Hirshman<sup>1</sup>, E.I. Ginns<sup>1</sup>.* 1) Brudnick Neuropsych Res Inst, Psychiatry Dept, Univ of Massachusetts Med Sch, Worcester, MA; 2) Childrens Hospital of Eastern Ontario and Univ of Ottawa, Ottawa, Ont. Canada.

Ellis-van Creveld (EvC) syndrome is an autosomal recessive disorder presenting with dwarfism, polydactyly, ectodermal dysplasia, congenital heart abnormalities (~60%) and rarely nephronophthisis. The absence of EVC gene mutations in some patients suggested genetic heterogeneity in this syndrome. Involvement of a second gene was demonstrated by identification of mutations in exons 17 and 18 of the contiguous EVC2 gene in an Ashkenazi individual with EvC, and subsequently confirmed by reports of mutations in Gypsy, Ecuadorian and Brazilian families. We now report mutations in both EVC and EVC2 in a 20-month-old caucasian EvC patient of French and English Canadian origins. The proband has marked shortening of the extremities (but normal chest circumference), radiographic features compatible with EvC, mild facial dysmorphism (midface hypoplasia with a glabellar nevus flammeus), abnormal frenulae, peg teeth with abnormal enamel, absence of one index finger phalanx, bilateral 4th finger clinodactyly, bilateral post-axial polydactyly, nail hypoplasia, renal dysplasia and an unbalanced AVSD requiring a bidirectional Glenn shunt. The EVC gene (117.1 kb) has multiple RNAs up to ~7,500 nucleotides in length, has four transcription start sites, 21 coding exons, three alternative 3' untranslated regions and tissue specific alternative splicing. The EVC2 gene (166.4 kb) has multiple RNAs up to ~4,800 nucleotides in length, has one transcription start site, 21 coding exons, two alternative 3' untranslated regions and alternative splicing characterized by long and short variants of several exons. Sequence analysis of EVC and EVC2 in this patient showed a deletion in exon 8 and an acceptor splice site mutation of exon 20 in one allele of the EVC gene, and a donor splice site mutation of exon 13 of the EVC2 gene. Inheritance of these mutations was confirmed by sequence analysis of parental alleles. Thus, the possibility of mutations in either or both EVC and EVC2 must be considered in counseling for this disorder.

**The Mutation P392L of the Sequestosome 1 Gene in Paget's Disease of Bone is frequent in the French Population.**

*L Michou<sup>1, 7</sup>, JL. Laplanche<sup>2</sup>, P. Orcel<sup>3</sup>, P. Hilliquin<sup>5</sup>, P. Quillet<sup>6</sup>, T. Bardin<sup>1,3</sup>, E. Vicaut<sup>4</sup>, JM. Launay<sup>2</sup>, F.*

*Cornelis<sup>1,7</sup>.* 1) CLinical Genetics Unit, Lariboisière Hospital, Paris, France; 2) Bichemistry department, Lariboisière Hospital, Paris, France; 3) Rheumatology department, Lariboisière Hospital, Paris, France; 4) Clinical Research Unit, Lariboisière Hospital, Paris, France; 5) Rheumatology department, Sud-Francilien Hospital, Evry, France; 6) Biology department, Sud-Francilien Hospital, Evry, France; 7) Clinical Genetics Unit, Sud-Francilien Hospital, Evry, France.

**Purpose.** Paget's disease of bone (PDB) is a late onset chronic disease of the skeleton that affects 3 % of the Caucasian population aged over 55 years. Paget's lesions exhibit an excess of bone turn-over and can be treated by biphosphonates, which inhibit osteoclast resorption. PDB often segregates as an autosomal dominant trait. Genetic heterogeneity has been demonstrated, with 7 PDB loci reported. At the PDB3 locus on chromosome 5, Laurin et al. (1) identified the first of Paget's genes, sequestosome 1 (SQSTM1), part of the RANK (Receptor Activator of NF-kB) pathway involved in osteoclast regulation. A recurrent mutation (P392L) was found in 16 % of the sporadic PDB patients and 46 % of the familial cases in the French Canadian population. This mutation was also found in the British population, in 9 % of sporadic and 19 % of familial cases, as well as two additional mutations: E396X and IVS7+1 G-A in 6 % and 1 % of the familial cases, respectively. Genetic testing in families carrying the PDB mutation is clinically relevant, leading to early radiological diagnosis at the clinically asymptomatic stage, when complications can be prevented by early treatment. The aim of this study was to evaluate the frequency of those SQSTM1 mutations in the French Caucasian population.

**Patients and methods.** Nineteen French Caucasian patients with sporadic PDB underwent genetic testing. The search for the P392L mutation relied on a Sac-I PCR-RFLP assay. Sequencing was performed for the two others mutations.

**Results.** Only the P392L mutation of SQSTM1 was detected, in 2 patients out of 19 (11%).

**Conclusion.** The P392L mutation of the SQSTM1 gene is frequent in the French Caucasian PDB population. Genetic testing of PDB patients in France is indicated, aiming at early diagnosis to prevent complications in relatives of patients carrying the mutation. (1) Laurin et al. *Am J Hum Genet* 2002 ; 70 (6) : 1582-8.

**Analysis of *FLNA* as a candidate gene for Terminal Osseous Dysplasia with Pigmentary Defects and Focal Dermal Hypoplasia.** *D. Sharma*<sup>1</sup>, *C. Bacino*<sup>2</sup>, *I.B. Van den Veyver*<sup>1, 2</sup>. 1) Obstetrics and Gynecology; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

We previously described a novel, male-lethal X-linked dominant developmental disorder, TODPD (Terminal Osseous Dysplasia with Pigmentary Defects), and mapped the candidate locus to Xq27.3-qter. Affected females have distinct facial anomalies in conjunction with skeletal (limb) deformities and skin pigmentation defects. This phenotype overlaps with that of Goltz syndrome (Focal Dermal Hypoplasia; FDH), another X-linked dominant disorder with limb anomalies and skin defects. A recent report describes missense and in-frame deletions in exons 3, 5, 11, 22, and 29 of the *FLNA* gene in four congenital malformation disorders (OPD1, OPD2, MNS, and FMD) with skeletal and limb abnormalities. *FLNA*, encoding a 280 kDa protein (Filamin A) that regulates re-organization of the actin cytoskeleton, comprises 48 coding exons and localizes to the candidate region of TODPD in Xq28. To test *FLNA* as a candidate gene for TODPD and FDH, we performed PCR amplification followed by direct sequencing of exons 3, 5, 11, 22, and 29 from peripheral blood leukocyte or lymphoblast derived DNA of 8 unrelated FDH patients and 2 TODPD females from a previously reported large multigenerational pedigree. An unaffected female from the TODPD family and an anonymous random male and female sample were included as normal controls. Results so far do not reveal any mutations in these exons in TODPD and Goltz patients. Analysis of the other coding exons of *FLNA* in these patients is in progress.

**Mutational, histologic and gene expression analysis in spondyloepiphyseal dysplasia tarda (SEDL).** *G.E. Tiller<sup>1</sup>, C. Baird<sup>1</sup>, T. Aigner<sup>2</sup>*. 1) Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN; 2) Department of Pathology, University of Erlangen-Nuremberg, Erlangen, Germany.

Spondyloepiphyseal dysplasia tarda (SEDL) is an X-linked skeletal dysplasia characterized by disproportionate short stature and early-onset osteoarthritis. The SEDL gene, *sedlin*, is located at Xp22.2, and a transcribed retropseudogene exists on chromosome 19. Mutational analysis of *sedlin* in SEDL patients has revealed over 25 unique mutations, including a recurrent splice mutation (IVS3+5GA) and a hot spot for recurrent 2-5bp deletions in exon 5. With rare exceptions, all mutations are predicted to generate premature stop codons and truncate the protein product. Immunohistochemical analysis of residual articular SEDT cartilage revealed normal staining for cartilage collagens. Real-time RT-PCR experiments failed to demonstrate nonsense-mediated mRNA decay of mutant *sedlin* transcripts. However, immunohistochemical analysis of cultured SEDL fibroblasts, using an anti-*sedlin* antibody, demonstrated no staining above background, compared to controls. These data suggest that truncated *sedlin* proteins are unstable and are degraded, and that the pseudogene, while transcribed, is not translated. We hypothesize that the pathophysiology of SEDL may involve an intracellular pathway previously unappreciated in chondrocytes, whose dysfunction interferes with extracellular matrix homeostasis.

**Hallervorden Spatz syndrome (HSS) in two families from southern Italy: a clinical and genetic study.** *I. Cirò*

*Candiano<sup>1</sup>, F. Annesi<sup>1</sup>, D. Civitelli<sup>1</sup>, P. Tarantino<sup>1</sup>, P. Spadafora<sup>1</sup>, S. Carrideo<sup>1</sup>, E.V. De Marco<sup>1</sup>, F. Naso<sup>3</sup>, D. Consoli<sup>3</sup>, A. Quattrone<sup>1,2</sup>, G. Annesi<sup>1</sup>.* 1) Inst of Neurol Sciences, National Research Council, Mangone, Cosenza, Italy; 2) Institute of Neurology, University of Magna Graecia, Catanzaro, Italy; 3) Azienda Ospedaliera, Vibo Valentia, Italy.

**Background and Objective.** Hallervorden Spatz syndrome (HSS) is an autosomal recessive disorder characterized by dystonia, parkinsonism, and iron accumulation in the brain. Many patients with HSS have mutations in the gene encoding pantothenate kinase2 (PANK2) located in chromosome 20p13. The aim of the current study was to investigate patients with HSS for mutations in the PANK2 gene. **Material and Methods.** Patients with HSS were classified as having the classic disease (characterized by early onset with rapid progression) or atypical HSS (later onset with slow progression). Primers were designed to amplify each of the seven exons of PANK2. Polymerase-chain-reaction-amplified DNA was sequenced in both the forward and the reverse directions and compared with control DNA. **Results.** One patient from the first family was affected by classic disease while two individuals in the other family presented with atypical disease. In all patients T2-weighted magnetic resonance imaging of the brain showed a specific pattern of hyperintensity within the hypointense medial globus pallidus. A novel mutation in the PANK2 gene in homozygous state was identified in two patients from the family with atypical HSS. This mutation was a missense mutation (C1069T) which resulted in an amino acid substitution (Arginine to Tryptophane at position 357), and was not found in 100 control chromosomes. No mutation in the coding regions of the PANK2 gene was found in the family with classic HSS. **Conclusion.** We identified a new mutation in the PANK2 gene in two patients with atypical HSS whereas no mutation was found in one patient with classic HSS. Our findings suggest that classic form of HSS is not always due to mutations in the coding region of the PANK2 gene.

**Agenesis of the corpus callosum and frontonasal dysplasia associated with a balanced translocation that disrupts the *FLJ21877* gene.** *J.J. Dowling*<sup>1</sup>, *M. Kato*<sup>2</sup>, *J. Chung*<sup>2</sup>, *D.K. Manchester*<sup>3</sup>, *E. Sujansky*<sup>3</sup>, *D.H. Ledbetter*<sup>2</sup>, *W.B. Dobyns*<sup>2</sup>. 1) Neurology, CHOP, Philadelphia, PA; 2) Human Genetics, U of Chicago, Chicago, IL; 3) Genetics, Children's Hospital, Denver, CO.

Agenesis of the corpus callosum (ACC) is a common human brain malformation, with an incidence estimated at 0.25 per 1000 livebirths. It can occur in isolation or be associated with other brain and somatic abnormalities. Among the latter, craniofacial anomalies are particularly common. While many different syndromes with ACC are known, fewer than ten causative genes have been identified.

Here we describe disruption of a novel gene in a male patient with ACC, frontonasal dysplasia, and a balanced translocation t(2;15)(p21;q14). Clinical evaluation documented mild mental retardation, hypertelorism, broad forehead, midfacial hypoplasia, prominent jaw, radioulnar synostosis, and mild webbing of fingers and toes. The father has a similar facial appearance, but chromosome analysis was not performed. Physical mapping of the translocation using FISH, southern blot analysis and PCR from somatic cell hybrids led to localization of the 2p breakpoint within an intron of a previously uncharacterized gene designated *FLJ21877*. The 15q breakpoint was localized to a region that contained no known or predicted genes. *FLJ21877* is a gene of unknown function that was initially identified as a large cDNA highly enriched in brain (Nagasi et al., DNA Res 2000;7:347). It codes for an approximately 2 kb transcript with 12 alternatively spliced exons that span greater than 300 kb of genomic DNA. Given the location of the breakpoint within the coding region, we believe alteration of *FLJ21877* expression is likely responsible for the abnormal phenotype in this patient via either haploinsufficiency or a dominant negative effect. *FLJ21877* is therefore one of the first genes implicated in ACC associated with frontonasal dysplasia.

**APRATAXIN, THE CAUSATIVE GENE FOR EARLY-ONSET ATAXIA WITH OCULAR MOTOR APRAXIA AND HYPOALBUMINEMIA (EAOH), IS A MEMBER OF SSDNA REPAIR MACHINERY. -IMPLICATION OF IMPAIRED DNA REPAIR SYSTEMS IN NEURODEGENERATION-. H. Date<sup>1</sup>, S. Igarashi<sup>1</sup>, T. Takahashi<sup>1</sup>, H. Takano<sup>1</sup>, O. Onodera<sup>1</sup>, M. Nishizawa<sup>1</sup>, Y. Sano<sup>2</sup>, S. Tsuji<sup>3</sup>. 1) Dept Neurology, BRI Niigata Univ, Niigata, Japan; 2) Dept Neurology, Yamaguchi Univ, Yamaguchi, Japan; 3) Dept Neurology, Univ of Tokyo, Tokyo, Japan.**

Early-onset ataxia with ocular motor apraxia and hypoalbuminemia (EAOH/AOA1) is one of the most frequent form of autosomal recessive spinocerebellar ataxias in the Japanese population. This disease has unique clinical presentations characterized by ocular motor apraxia, cerebellar ataxia, peripheral neuropathy and hypoalbuminemia. Interestingly, some of these clinical presentations overlap with those of Friedreich's ataxia and ataxia-telangiectasia. By a positional cloning approach, we have recently identified the causative gene for EAOH/AOA1 and designated as aprataxin (APTX). Aprataxin has two major mRNA species as a result of alternative splicing of exon 3. Although both forms of aprataxin mRNAs are expressed at comparable levels in various human tissues, the long form of APTX (LAPTX) has been found to be a major form in human central nervous system based on Western blot analysis. These results suggest that the loss of function of LAPTX underlies the neurodegenerative processes in this disease. The N-terminus portion of LAPTX shows a homology to the polynucleotide kinase-3'phosphatase (PNKP), which plays an important role in single-strand DNA (ssDNA) break repair in collaboration with XRCC1, DNA polymerase  $\beta$ , poly (ADP-ribose) polymerase and DNA ligase III. Here we demonstrate that long form APTX interact with XRCC1 in a yeast two-hybrid system and a co-immunoprecipitation experiment. Furthermore, poly (ADP-ribose) polymerase has been shown to co-immunoprecipitate with LAPTX, but not with the short form of APTX. These observations strongly suggest that the LAPTX is involved in single strand DNA break repair as a member of a complex together with XRCC1 and poly (ADP-ribose) polymerase, and that the gradual accumulation of DNA damages underlies the neuronal dysfunction in EAOH/AOA1.

**Microarray analysis of compensatory changes in gene expression in Frataxin deficient mouse brain.** *S-H. Choi<sup>1</sup>, D. Tentler<sup>1</sup>, M.M. Santos<sup>2</sup>, M. Pandolfo<sup>2</sup>, D.H. Geschwind<sup>1</sup>*. 1) Program in Neurogenetics, Department of Neurology, David Geffen School of Medicine at UCLA, Los Angeles, CA; 2) Department of Medicine, Centre Hospitalier de le Universite de Montreal, Hopital Notre-Dame, Montreal, PQ, Canada.

Friedreichs ataxia is one of the most common forms of autosomal recessive ataxia and is caused by reduced expression of the frataxin gene. The clinical features include neurological, cardiovascular, skeletal and endocrine abnormalities. Frataxin deficiency leads to iron accumulation within mitochondria, increasing free radical production by fenton chemistry, and altering oxidative metabolism. The direct pathway(s) by which this results in cell death are not known. We applied microarray analysis of gene expression to identify specific pathways affected by reduced frataxin level, which lead to neurodegeneration in Friedreichs ataxia. To identify the early alterations caused by frataxin deficiency and the cellular compensatory response to this stress, we analyzed gene expression in mice expressing 25 to 36% of wild-type frataxin levels, which have no observable neurological phenotype or neuropathology (Miranda et al., 2002). A custom 10K-element cDNA microarray was probed with cDNA from 4 replicates of 3 brain regions affected in Friedreichs ataxia. We identified upregulation of a number of genes in a variety of signaling pathways and biological processes. The absence of neurodegeneration in these mice has allowed us to identify the specific cellular response to frataxin deficiency, rather than detecting changes due to cell loss or inflammation. In this context, the differentially expressed genes obtained in our study likely reflect the true cellular dysregulation and compensation for frataxin loss, as the cell struggles for continued survival. These putative compensatory pathways become novel therapeutic targets.

**Molecular studies of chorein, the protein altered in chorea-acanthocytosis.** C. Dobson-Stone, A. Velayos, A.P. Monaco. Wellcome Trust Centre for Human Genetics, Oxford University, Roosevelt Drive, Oxford OX3 7BN, UK.

Chorea-acanthocytosis (ChAc) is an autosomal recessive neurological disorder, whose characteristic features include hyperkinetic movements and abnormal red blood cell morphology, caused by alteration of the *CHAC* gene on 9q21. To date, 71 different mutations, distributed throughout the gene, have been reported. *CHAC* encodes a large, novel protein called chorein. It shares significant homology with the yeast protein Vps13p, implicated in protein sorting. No known localisation signals, structural motifs or domains are detected in this protein. We have undertaken the functional characterisation of chorein, focussing our efforts on i) its subcellular localisation and ii) the identification of proteins that interact with it.

i) An N-terminal portion of chorein, overexpressed in bacteria as a glutathione-S-transferase-fusion protein and then cleaved and purified, was used to raise a polyclonal antibody (anti-chor1) in rabbit. Using Western blot analysis and immunofluorescence techniques, we have shown that this antibody detects chorein. To determine the subcellular location of chorein, we tagged chorein with myc, FLAG or EGFP epitopes. Chorein was visualised using immunofluorescent labelling in a number of mammalian cell lines after transient (cos-1, HEK293, HeLa and MRC5) or stable (HEK293) transfection, and it was detected in vesicular-like structures.

ii) In order to identify proteins that may interact with chorein, we have performed yeast two-hybrid analyses using different regions of chorein as baits. We have identified several putative interactors, including MAP kinase kinase kinase 7 interacting protein 2 (MAP3K7IP2, TAB2). Co-immunoprecipitation and GST-pulldown techniques will be used in the further analysis of this interaction.

These studies provide the first steps in the functional characterisation of chorein that will eventually provide some clues as to how the absence of chorein leads to the clinical features seen in ChAc.

**Prevalence of SCA14 and spectrum of PKC mutations in a large panel of ataxia patients.** *D.H. Chen<sup>1</sup>, P.J. Cimino<sup>2</sup>, L. Ranum<sup>4</sup>, I. Yabe<sup>5</sup>, H. Sasaki<sup>5</sup>, M. Matsushita<sup>2</sup>, T.D. Bird<sup>1,3</sup>, W.H. Raskind<sup>2,3</sup>.* 1) Depts of Neurology and; 2) Medicine, Univ Washington; 3) MIRECC, SVAMC, Seattle, WA; 4) Institute of Human Genetics, Univ Minnesota; 5) Dept of Neurology, Hokkaido Univ Graduate School of Medicine, Japan.

SCA is a group of hereditary neurodegenerative disorders with at least 20 autosomal dominant forms. Nine of the 10 SCAs for which the genes are known result from nucleotide expansions. We recently discovered a new genetic mechanism for SCA mutations in protein kinase C gamma (*PRKCG*; PKC; MIM 179680), a serine/threonine kinase primarily expressed in the central nervous system. Of 89 SCA samples screened, four different mutations in *PRKCG* were identified in one sporadic case and three familial cases, including the Japanese family in which SCA14 was originally described. To investigate the prevalence of SCA14, the spectrum of mutations in *PRKCG*, and genotype-phenotype correlations, we screened a large collection of samples from ataxia patients who were negative for SCA DNA expansions. Of the 236 samples, 78 were familial, 134 were apparently sporadic and 24 were not specified. Because all previously detected mutations were clustered in exon 4, we speculated that it may be a hotspot for mutations in SCA14. Therefore, we initiated the screening with exon 4. A six base-pair deletion, predicting omission of lysine 100 and histidine 101, was found in a sporadic case. The mutations all affect highly conserved residues in the regulatory region of the gene (H101Y, S119P, Q127R, G128D, and del. 100K-101Y). Axial myoclonus was a feature in early onset cases in the Japanese family, but all other affected individuals manifested uncomplicated ataxia. To date, exon 4 mutations in *PRKCG* account for 3/149 familial and 2/176 sporadic or unknown ataxias without an identified repeat expansion. Although SCA14 represents a small fraction of ataxia cases, it is reasonable to sequence exon 4 in this subset of ataxia patients. Ongoing screening of the remaining 17 exons will further our understanding of the genotype-phenotype correlations and the functional role of PKC in SCA14. These new results will be presented.

**A splice-junction mutation in Sbf2 gene causes autosomal recessive Charcot-Marie-Tooth disease (CMT4B2) in a family from southern Italy.** *F. Conforti<sup>1</sup>, R. Mazzei<sup>1</sup>, A. Patitucci<sup>1</sup>, P. Valentino<sup>2</sup>, A. Magariello<sup>1</sup>, T. Sprovieri<sup>1</sup>, F. Bono<sup>2</sup>, J. Senderek<sup>3</sup>, A. Gabriele<sup>1</sup>, G. Peluso<sup>1</sup>, R. Nistico<sup>2</sup>, C. Bergmann<sup>3</sup>, M. Muglia<sup>1</sup>.* 1) ISN-CNR, Mangone, Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro, Italy; 3) Department of Human Genetics, Aachen University of Technology, Aachen, Germany.

Autosomal recessive Charcot-Marie-Tooth disease type 4 (CMT4) comprises a group of clinically and genetically heterogeneous disorders of the peripheral nervous system. At least 10 loci are responsible for autosomal recessive CMT and six genes have identified so far. In this study, we report a small pedigree with a recessive form of CMT (CMT4B) from Southern Italy in which the linkage to chromosome 11q23 was excluded. There were six individuals in two generations with two affected subjects. We performed haplotype analysis using highly polymorphic microsatellite markers located on chromosome 11p15. Subsequently, the coding region of the Sbf2 gene was sequenced by using primers flanking intron-exon boundaries. Using 7 microsatellite markers located on chromosome 11 (D11S4186, D11S909, D11S932, D11S4149, D11S1329, D11S1999 and D11S1346), the haplotype analysis revealed a suggestive linkage to chromosome 11p15. Direct sequencing of the Sbf2 gene showed a GC exon 32/intron 32 splice-junction mutation. In the current study we identify a splicing mutation in SBF2 gene in a small family with CMT4B linked to chromosome 11p15. Mutational screening of Sbf2 revealed a homozygous mutation in the splice-junction donor-acceptor site of exon 32 (+1GC) in the affected patients. The variation was also confirmed by digestion with restriction enzyme Alu I, which cleaved the wild-type PCR product of 370 bp into 210 bp and twice 80 bp digestion fragments. The corresponding region of the Sbf2 gene in our affected patients was cleaved into 210 bp, 80 bp, 68 bp and 12 bp digestion fragments. This mutation was absent in 100 control chromosomes examined. This is the first finding of a mutation in the Sbf2 gene that alters the correct splicing of the gene. Furthermore, these data confirm that mutations in the Sbf2 gene are causative of CMT4B2.

**Generation and characterization of mouse models of spastic paraplegia caused by mutation of SPG4 gene. C.**

*Fassier<sup>1</sup>, A. Tarrade<sup>1</sup>, D. Charvin<sup>1</sup>, S. Betuing<sup>1</sup>, N. Fonknechten<sup>2</sup>, G. Millet<sup>1</sup>, S. Courageot<sup>1</sup>, N. Roblot<sup>1</sup>, A. Dierich<sup>3</sup>, J. Melki<sup>1</sup>.* 1) Molecular Neurogenetics Laboratoty, INSERM E-223, Evry, France; 2) Genoscope, CNS, Evry; 3) IGBMC, Illkirch, France.

Hereditary spastic paraplegia (HSP) is a progressive neurodegenerative disorder characterized by axonal degeneration of corticospinal tracts and posterior columns. The most prevalent form of autosomal dominant HSP is linked to the SPG4 locus encoding spastin (Sp), a member of the AAA protein family. In order to elucidate the pathophysiology of HSP, mouse models have been generated. A targeting vector that includes two loxP sites flanking exons 5 to 7 of the murine Sp gene (Spf) and a NeoR gene was constructed. Through homologous recombination, (Spf/+) heterozygous mice were created. To generate mice carrying heterozygous deletion of Sp exons 5 to 7 in all cell types, (Spf/+) mice were crossed to CMV-Cre transgenic mice. Mice heterozygously deleted in all cell types (Spdel/+) are viable and fertile. Transcript analysis revealed a marked reduction of full-length (FL) Sp transcripts in brain of mutant mice associated with truncated transcripts lacking exons 5 to 7 when compared to control. Surprisingly, less than the expected half dose of FL Sp transcripts was observed in brain of (Spdel/+) mice when compared to that found in other mutant tissues. To determine whether HSP was caused by defect of Sp in neurons, transgenic mice expressing the Cre recombinase transgene under the control of the NSE promoter were used (NSE-Cre). Using Rosa 26 reporter mice, Cre-mediated activity of -galactosidase was observed in spinal cord and in several regions of the brain including motor cortex, the region affected in HSP. (NSE-Cre,Spf/+) mice were generated and transcript analysis of Sp revealed results similar in brain to those found in (Spdel/+) mice. Molecular analysis of these two mice models suggest that, in brain, Sp could play a direct or indirect role on its transcription. Further characterization of both types of mutant mice including behavioural and morphological studies should allow us to validate mutant mice as mouse models of HSP and should provide new insights into pathogenic mechanisms leading to HSP.

**LMNA, MTMR2 and GDAP1 : implication in autosomal recessive Charcot-Marie-Tooth disease.** A. DeSandre-Giovannoli<sup>1</sup>, T. Hamadouche<sup>2</sup>, R. Bernard<sup>3</sup>, M. Tazir<sup>4</sup>, M. Chaouch<sup>5</sup>, I. Boccaccio<sup>1</sup>, V. Delague<sup>6</sup>, JM. Vallat<sup>7</sup>, C. Desnuelle<sup>8</sup>, A. Megarbane<sup>6</sup>, D. Grid<sup>9</sup>, N. Levy<sup>1,3</sup>. 1) INSERM U491, Genetique Medicale et Developpement, Marseille, France; 2) Institut Pasteur d'Algerie, Algiers; 3) Departement de Genetique Medicale, Hopital Timone, Marseille, France; 4) Service de Neurologie, CHU Ben-Aknoun, Algiers; 5) Service de Neurologie, CHU Mustafa, Algiers, Algeria; 6) Service de Genetique, Universit Saint-Joseph, Beyrouth, Lebanon; 7) Service de neurologie, Hopital l'Archet, Nice, France; 8) Neuropathologie, CHU Dupuytren, Limoges, France; 9) Genethon III, Evry, France.

Charcot-Marie-Tooth (CMT) disorders are the most frequent inherited peripheral neuropathies. Electrophysiological criteria serve to classify patients as presenting demyelinating (CMT1), axonal (CMT2) or intermediate neuropathies. Autosomal recessive forms are less frequent, more severe and early onset forms. We report homozygous mutations in LMNA, MTMR2 and GDAP1 causing respectively axonal (CMT2B1), demyelinating (CMT4B1) and mixed (CMT4A) neuropathies in families affected with autosomal recessive CMT. We identified the LMNA c.892C>T founder mutation segregating in 11 Algerian families affected with axonal CMT. MTMR2 sequencing revealed a nonsense change and a 1bp insertion, respectively in two families affected with a demyelinating phenotype. Finally, GDAP1 splicing and nonsense mutations in Algerian and Lebanese families, caused very severe mixed CMT. LMNA encodes Lamins A/C, major structural proteins of nuclear laminae. MTMR2 encodes a member of the myotubularin phosphatase multigenic protein family, with specific activity towards phosphatidylinositol 3-phosphate. On the other hand, GDAP1 structure and expression pattern locate it at the crossroads between gangliosides and Gluthatione S-Transferases diverse functions. While GDAP1 is mainly expressed in nervous tissues, LMNA and MTMR2 are ubiquitously expressed. Additionally, different mutations in LMNA cause a series of only partially related hereditary disorders. Unraveling the functional interplays underlying the pathophysiology of CMT diseases is today the main issue, focusing on the structure-function-phenotype relationships.

**Mutation analysis of the TSC2 gene in 23 TSC families from India.** *M. Ali*<sup>1</sup>, *M. Markandaya*<sup>1</sup>, *S.C. Girimaji*<sup>2</sup>, *A.K. Shukla*<sup>3</sup>, *S. Sacchidanand*<sup>4</sup>, *A. Kumar*<sup>1</sup>. 1) MRDG, Indian Institute of Science, Bangalore, Karnataka, India; 2) Dept. of Psychiatry, NIMHANS, Bangalore, India; 3) Department of Radiology, KIMS, Bangalore, India; 4) Department of Dermatology, Bangalore Medical College, Bangalore, India.

Tuberous sclerosis complex (TSC) is an autosomal dominant neuro-cutaneous disorder with loci on chromosome 9q34.3 (TSC1) and chromosome 16p13.3 (TSC2). Genes for both loci have been isolated and characterized. Pathologically, tuberous sclerosis complex is a disorder of cell proliferation, differentiation and migration. Clinical symptoms of TSC include cortical tubers, subependymal nodules, mental retardation, seizures, autism, shagreen patches and angiofibromas on the skin, cardiac rhabdomyomas, retinal hamartomas, ungual and peringual fibromas, and cysts and angiomyolipomas in the kidneys. More than 350 mutations have been reported in both TSC genes in patients mainly from the western and Japanese populations. However, there is no report on the mutation analysis of TSC genes in patients from the Indian population. We report here the mutational analysis of the TSC2 gene in 23 TSC families from India. Using PCR-SSCP and DNA sequence analyses, we have screened all 41 exons and promoter region of the TSC2 gene in seven familial and 16 sporadic TSC cases. We have identified a total of 12 mutations representing 25% and 75% of familial and sporadic cases respectively. Of these, eight mutations are novel. Of 12 mutations, 3 are deletions, 2 are insertions, 4 are missense, 2 are splice site and 1 is a nonsense mutation. In addition, we have also detected nine single-nucleotide polymorphisms (SNPs) in the TSC2 gene. Of these, three are novel SNPs. There was no correlation between the types of mutations (missense, nonsense, etc.) and the severity of the disease. The mutations were distributed across the gene without any clustering, suggesting that mutation analysis requires scanning of the complete coding sequence of the TSC2 gene (This work was financially supported by a grant from DBT, New Delhi to AK and SCG and a CSIR JRF to MA).

**The SPG3 disease gene atlastin: new mutations and the characterization of its murine orthologue.** A. Abel<sup>1</sup>, A. Hofer<sup>1</sup>, D. Del Turco<sup>2</sup>, S. Klimpe<sup>3</sup>, T. Deller<sup>2</sup>, G. Auburger<sup>1</sup>. 1) Section Molecular Neurogenetics, Department of Neurology, Johann Wolfgang Goethe University, Frankfurt/Main, Germany; 2) Institute for Clinical Neuroanatomy, Department of Neurology, Johann Wolfgang Goethe University, Frankfurt/Main, Germany; 3) Department of Neurology, Johannes Gutenberg University, Mainz, Germany.

Mutations in the large GTPase atlastin cause autosomal dominant spastic paraplegia. Atlastin is encoded in 15 exons and comprises 558 amino acids. In cooperation with clinical specialists around Germany we have performed a mutation screening by sequencing all coding exons and adjacent intron sequences in samples from 22 individuals or pedigrees, and identified two novel missense mutations. The F151S (ttt tct, exon 5) mutation, just two amino acids downstream from the conserved DxxG motif of the GTPase domain, was found in a 4-generation pedigree with DNA samples available from 10 affected and 7 unaffected individuals. A new MnlI restriction site resulting from this mutation was used to confirm complete cosegregation of the spastic paraplegia phenotype with the F151S mutation in this family and to exclude the mutation from 100 normal control chromosomes. The I315S (atc agc, exon 10) mutation was found in an affected individual with a positive family history, yet access to DNA samples of other family members could not be obtained.

We have also cloned and sequenced the murine Atlastin full-length cDNA. In situ hybridization of murine brain sections revealed atlastin expression in selected neuronal, but not glial cell populations. We will also present subcellular localization data derived from our current immunohistochemical studies of murine brain with a polypeptide antibody against a carboxy-terminal epitope.

**SOD1 Gene Mutations in Amyotrophic Lateral Sclerosis Italian Patients.** *C. Gellera<sup>1</sup>, D. Testa<sup>1</sup>, P. Passariello<sup>1</sup>, R. Mineri<sup>1</sup>, L. Morandi<sup>1</sup>, M. Sabatelli<sup>4</sup>, E. Munerati<sup>2</sup>, M. Corbo<sup>3</sup>, C. Mariotti<sup>1</sup>, V. Silani<sup>3</sup>, S. Di Donato<sup>1</sup>, F. Taroni<sup>1</sup>.* 1) Istituto Nazionale Neurologico IRCCS Carlo Besta, Milano, Italy; 2) Ospedale San Raffaele, IRCCS, Milano, Italy;; 3) Istituto Auxologico Italiano IRCCS, University of Milan Med. School, Milano, Italy; 4) Universit del Sacro Cuore, Roma, Italy.

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disorder (incidence 1:100.000). Only a small percentage of patients (10%) shows an inherited form. Molecular studies have allowed to identified at least 8 loci (ALS1, 2, 4, 5, 6, ALSX, ALS-FTD and ALS-NFHC). The causative gene has been identified in 3: ALS1-SOD1, ALS2-Alsin and ALS-NFHC. Mutations in the SOD1 gene are present in 10-20% of familial cases with more than hundred mutations described so far. Our work has been focused to the identification of mutations in SOD1 gene in a cohort of Italian ALS patients. Previous studies have shown that the disease progression may be due by the specific SOD1 mutation, although a great variability in disease duration has been observed among and within families with the same mutation. A great variability in the percentage and distribution of SOD1 specific mutations has also been observed in populations with different ethnic backgrounds. We have screened 310 Italian patients including 54 familial cases and found mutations in 12/54 (22%) unrelated families and in 4/256 (1.5%) sporadic patients. Eight FALS patients carried previously described mutations in heterozygous form: A4V (4 families), L84F (3 families), and G93D (1 family), while the remaining 4 FALS patients carried different new mutations. In two sporadic patients we have found two previously described SOD1 gene mutations: the homozygous D90A mutation and the heterozygous I113T, respectively. In the two additional sporadic cases we have found new heterozygous mutations: A95T and V97L. It is interesting to note that 58% (7/12) of SOD1 mutated Italian FALS patients harbour two prevalent mutations. A subset of patients (95/310) have also been screened for deletions in SMN2 gene: we do not have found a significative number of patients with deletions respect to controls.

**A reduced activity of the Na-K ATPase is responsible for Familial Hemiplegic Migraine.** *G. Casari<sup>1</sup>, M. De Fusco<sup>1</sup>, P. Aridon<sup>1</sup>, L. Silvestri<sup>1</sup>, L. Atorino<sup>1</sup>, L. Rampoldi<sup>1</sup>, A. Ballabio<sup>3</sup>, R. Marconi<sup>2</sup>.* 1) Dept. of Neuroscience, San Raffaele Scientific Institute, Milan, Mi, Italy; 2) Div. of Neurology, Misericordia Hospital, Grosseto, Italy; 3) TIGEM, Naples, Italy.

Headache attacks and autonomic dysfunctions characterize migraine, a very common disabling disorder with a 12 percent prevalence in the general population of western countries. Approximately 20% of migraineurs experience aura, a visual or sensory-motor neurological dysfunction, which usually precedes or accompanies the headache. Although the mode of transmission is controversial, population-based and twin studies indicate that genetic factors are implicated, prevalently in migraine with aura. Familial hemiplegic migraine (FHM) is a hereditary form of migraine with aura and some degree of hemiparesis. Two chromosomal loci are associated to FHM: FHM1, on chromosome 19, and FHM2 on chromosome 1q21-23. Mutations of the alpha-1A subunit of the voltage gated calcium channel (CACNA1A) are responsible for FHM1. We narrowed the FHM2 locus by linkage analysis on two large Italian families affected by pure FHM. The new critical region covers a small area of 0.9 Mb in 1q23 and renders feasible a positional candidate approach. Furthermore, we demonstrate that mutations of the alpha 2 subunit of the Na,K pump are responsible for the familial hemiplegic migraine form associated to chromosome 1q23 (FHM2, MIM 602481). Functional data show that a putative pathogenetic mechanism is triggered by a loss-of-function of a single allele of the Na,K pump.

**Aprataxin mutations are not a significant cause of recessive ataxia in the Mexican Mestizo population. M.**

*Gomez*<sup>1</sup>, *L. Hern*<sup>1</sup>, *T. Ashizawa*<sup>2</sup>, *E. Alonso*<sup>3</sup>, *A. Rasmussen*<sup>3</sup>, *S.I. Bidichandani*<sup>1</sup>. 1) Dept Biochem & Molec Biol, Oklahoma Univ Health Sci Ctr, Oklahoma City, OK; 2) Neurology, UTMB Galveston, TX; 3) National Institute of Neurology, Mexico City, Mexico.

Friedreich ataxia is the most common recessive ataxia, accounting for 75% of all recessive ataxias among Indo-Europeans. East Asians and Native Americans do not develop Friedreich ataxia because they lack the mutagenic *FRDA* alleles that cause the abnormal GAA triplet-repeat expansion seen in the vast majority of patients. Recently it was shown that the most common cause of recessive ataxia in Japan includes loss-of-function mutations in the *APTX* gene that encodes aprataxin. *APTX* mutations were also shown to be the second most common cause of recessive ataxia in Portugal. We have previously shown that Friedreich ataxia accounts for approximately 10% of all recessive ataxias in the Mexican Mestizo population and that this is largely due to the preponderance of non-mutagenic Native American *FRDA* genes among this population. Given the phenotypic overlap between patients with *APTX* and *FRDA* gene mutations, as exemplified by the Japanese and Portuguese studies, we decided to evaluate the role of *APTX* mutations as a cause of recessive ataxia in the majority of Mexican Mestizo patients without *FRDA* mutations. We screened all seven exons of the *APTX* gene in 90 Mexican Mestizo recessive ataxia patients who had previously tested negative for Friedreich ataxia and found no disease causing mutations. A parallel analysis of 13 Caucasian recessive ataxia patients referred for Friedreich ataxia testing, but who tested negative for *FRDA* mutations, revealed one patient who was homozygous for the Portuguese founder *APTX* mutation (W279X). We detected two common polymorphisms previously described in the Portuguese population, consisting of deletions in poly(T) stretches preceding the acceptor splice sites of exons 4 and 6; 41.4% and 55.5% were heterozygous, respectively. These data indicate that *APTX* mutations are not a significant cause of recessive ataxia in the Mexican Mestizo population, and that the cause of their ataxia remains largely unknown.

Program Nr: 2224 from 2003 ASHG Annual Meeting

**Mutations in POMT1 are a rare cause of Walker Warburg Syndrome.** *S.C. Currier<sup>1</sup>, B.S. Chang<sup>1</sup>, C.K. Lee<sup>1</sup>, A. Hassan<sup>1</sup>, A.L. Bodell<sup>1</sup>, W.B. Dobyns<sup>2</sup>, C.A. Walsh<sup>1</sup>.* 1) Howard Hughes, Beth Israel Deaconess Medical Center, Department of Neurology, program in Neuroscience, and Harvard Medical School, Boston, MA, 02115, USA; 2) Department of Human Genetics, Neurology and Pediatrics, The University of Chicago, Chicago, IL, USA.

Walker Warburg Syndrome (WWS) is an autosomal recessive disorder of infancy diagnosed by hydrocephalus, agyria, retinal dysplasia, and congenital muscular dystrophy and characterized by over migration of cortical neurons. Previous work (D.B.Valero de Bernab et al, Am J Hum Genet, 2002) identified mutations in the o-mannosyl transferase, POMT1, in six out of thirty unrelated WWS families. In order to investigate the genetic cause of WWS we performed a 10 cm genome wide screen in seven additional families. None of these families demonstrate linkage to the POMT1 locus. We have begun sequencing the POMT1 gene in more than thirty new WWS patients. Our preliminary results have not yet revealed any POMT1 mutations suggesting that they are a rare cause of WWS. Supported by: NIH RO1 NS35129.

**Functional analyses of the *NIPA1* gene with dominant negative mutations in hereditary spastic paraplegia (SPG6) and mapping within the Prader-Willi/Angelman syndrome region.** *J-H. Chai*<sup>1</sup>, *S. Rainier*<sup>2</sup>, *J.K. Fink*<sup>2</sup>, *R.D. Nicholls*<sup>1</sup>. 1) Department of Psychiatry, University of Pennsylvania, Philadelphia, PA; 2) Department of Neurology, University of Michigan, Ann Arbor, MI.

Hereditary spastic paraplegias (HSPs) are a genetically heterogeneous group of disorders characterized by progressive lower limb spasticity and weakness, with degeneration of the long corticospinal tracts. Although 20 spastic gait (SPG) loci have been genetically mapped, only 8 causative genes have been identified, and the molecular pathogenesis is poorly understood. SPG6, an autosomal dominant HSP, maps proximally within chromosome 15q11-q13, a region associated with Prader-Willi/Angelman syndromes (PWS/AS). We have identified a missense mutation (T45R) in a novel non-imprinted gene within the PWS/AS region, *NIPA1*, as responsible for SPG6. PWS/AS patients with chromosome deletions are hemizygous for *NIPA1* but do not show an SPG6 phenotype, indicating that T45R is a dominant negative mutation. Previous studies showed that *NIPA1* is predicted to encode a polypeptide with 9 transmembrane (TM) domains, suggestive of function as a transporter or receptor, and is highly expressed in mammalian brain. Extensive phylogenetic analysis now indicates that a *NIPA1*-related superfamily occurs in vertebrates, invertebrates, plants and bacteria. Immunohistochemical studies using anti-Nipa1 antisera shows expression of mouse *NIPA1* in neurons of cortex, hippocampus and spinal cord. In HeLa cell fusion gene studies, Nipa1-EGFP is in cytoplasmic vesicles that co-localization with -Lamp1 shows are late endosomes/lysosomes. Western blot analyses using anti-*NIPA1* and anti-GFP antisera with total or membrane protein fractions from transfected HeLa cells or mouse tissues detected ~90 kD and ~70 kD isoforms of Nipa1-EGFP or an ~40 kD Nipa1 protein, respectively, with an ~23 kD processed isoform in brain. Membrane fraction enrichment in each case confirms *NIPA1* functions as a TM protein. Identification of the *in vivo* subcellular localization, ligand, and development of animal models will reveal the role of *NIPA1* in axonal maintenance and may lead to therapeutic approaches in the HSP neurodegenerative disorders.

**MRI studies in SCA8: Cerebellar atrophy in unaffected expansion carriers partially explains reduced penetrance.** *J.W. Day, H. Liu, J. Dalton, Y. Ikeda, L.P.W. Ranum.* Inst Human Genetics, MMC 206, Univ MN, Mpls, MN.

We previously reported that an untranslated CTG expansion causes spinocerebellar ataxia type 8 (SCA8). In the large MN-A family we initially characterized ( $Z=6.8$ ,  $=0.0$ ), affected individuals have longer repeats (mean=117 CTGs,  $n=13$ ) than unaffected carriers (mean=90 CTGs,  $n=21$ ,  $p=1 \times 10^{-8}$ ). The reduced penetrance seen in the MN-A family also occurs in other SCA8 families. The observation of SCA8 expansions on control chromosomes suggests that in addition to the CTG repeat expansion other environmental or genetic factors may play a role in disease pathogenesis. To better characterize SCA8, and to investigate the causes of reduced penetrance, we performed volumetric, functional and spectroscopic MRI studies of genetically normal, clinically affected and unaffected expansion carriers in the MN-A family. Volumetric studies show that cerebellar volume of clinically affected SCA8 subjects ( $n=4$ ) is ~50% of normal ( $n=4$ ; mean=57cm<sup>3</sup> vs. 116cm<sup>3</sup>), without cerebral or brainstem atrophy. Functional MRI studies show a dramatic loss of cerebellar but not cerebral response during repetitive finger-tapping. Spectroscopy shows a significant reduction in the choline:creatine ratio in cerebellum and cerebrum. Combined MRI data may prove useful in comparing disease characteristics in unrelated SCA8 families.

A clinically unaffected MN-A family member with 140 CTGs, well above the family's pathogenic threshold, had an entirely normal clinical exam; on repeated evaluation she was able to stand or hop on one foot for >30s, and to tandem walk backwards and forwards, all with her eyes closed. Surprisingly her MRI showed dramatically (~50%) reduced cerebellar volume (53cm<sup>3</sup>). Marked cerebellar atrophy has also been seen in other clinically unaffected MN-A and Japanese expansion carriers. The use of MRI screening is clearly more sensitive than neurological exams in assessing the pathogenic effects of the SCA8 CTG expansion and may explain some of the apparent reduced penetrance that has been reported. Remarkably, significant cerebellar atrophy is not necessarily associated with ataxia in this slowly progressive degenerative disease.

**Genomic multiplication of the alpha-synuclein gene in three kindreds with familial Lewy body disease. M.**

*Farrer<sup>1</sup>, D. Wang<sup>1</sup>, S. Lincoln<sup>1</sup>, J. Kachergus<sup>1</sup>, W.J. Langston<sup>2</sup>, D. Dickson<sup>1</sup>.* 1) Neuroscience, Mayo Clinic Jacksonville, Jacksonville, FL; 2) Parkinson's Institute, Sunnyvale, CA.

The Spellman-Muenter (Iowa) kindred, in which affected individuals present clinically with early-onset parkinsonism, has been followed by Mayo Clinic for >90 years. At autopsy, patients have diffuse Lewy body disease. Recent genome-wide linkage analysis identified alpha-synuclein as the locus that segregates with disease ( $z=3.65$ , D4S2460). Genomic triplication of a 2.1Mb region results in alpha-synuclein gene/protein over-expression (Singleton, Farrer et al., 2003). We now present genetic, clinical and pathological comparisons of this and two unrelated kindreds with familial Lewy body disease, in which genomic multiplication of alpha-synuclein locus is causal for disease. Haplotype analysis demonstrates that these families are independent and not related to a common ancestral founder. Our analysis serves to highlight the intrinsic genomic instability of the region. mRNA and protein studies from brain tissue also clearly demonstrate upregulation of alpha-synuclein gene and protein expression. Genomic, clinical and pathological correlates of disease, within these families will be compared. The profound implications for idiopathic Lewy body disorders will be discussed.

**A complex neurocristopathy results from dominant-negative *SOX10* mutations that escape nonsense mediated decay.** *K. Inoue*<sup>1</sup>, *M. Khajavi*<sup>1</sup>, *T. Ohyama*<sup>1</sup>, *S. Hirabayashi*<sup>2</sup>, *J. Wilson*<sup>3</sup>, *J.D. Reggin*<sup>4</sup>, *P. Mancias*<sup>5</sup>, *I.J. Butler*<sup>5</sup>, *M.F. Wilkinson*<sup>6</sup>, *M. Wegner*<sup>7</sup>, *J.R. Lupski*<sup>1</sup>. 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Div Pediatric Neurology, Nagano Children's Hospital, Nagano, Japan; 3) Dept Neurology, Great Ormond Street Children's Hospital, London, UK; 4) Meritcare Neuroscience Clinic, Fargo, ND; 5) Dept Neurology, Univ of Texas Houston, Houston, TX; 6) Dept Immunology, University of Texas M.D. Anderson Cancer Center, Houston, TX; 7) Institut für Biochemie, Universität Erlangen-Nürnberg, Erlangen, Germany.

We investigated whether mutations in an open reading frame can dictate disease severity by a mechanism other than affecting the protein product. In particular, we investigated whether mutations that trigger the nonsense-mediated decay (NMD) RNA surveillance pathway and thereby decrease mRNA levels can decrease disease severity. As a test case, we examined patients with mutations in the *SOX10* gene, who have either a complex neurological disease phenotype termed PCWH (peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome and Hirschsprung disease) or a more restricted phenotype termed Waardenburg-Shah syndrome. We found that while all nonsense mutations that prematurely terminate translation generate mutant truncated *SOX10* proteins with potent dominant-negative activity, the more severe disease phenotype is prevented by mutations in internal exons, as these mutations elicit the NMD pathway. Our findings suggest that NMD functions to mitigate the harmful effects of a major subset of truncating mutations and thus has the potential to moderate the symptoms of many genetic diseases.

**Characterization of the breakpoints of PLP1 duplication in three cases of Pelizaeus-Merzbacher disease. A.**

*Iwaki<sup>1</sup>, J. Kondo<sup>1</sup>, M. Ototsuji<sup>1</sup>, K. Kurosawa<sup>2</sup>, Y. Fukumaki<sup>1</sup>.* 1) Research Center for Genetic Information, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; 2) Kanagawa Children's Medical Center, Yokohama, Japan.

Duplication of the proteolipid protein gene (PLP1) on chromosome Xq22 causes Pelizaeus-Merzbacher disease (PMD), an X-linked disorder characterized by dysmyelination in the central nervous system. The size of the duplicated genomic segments varies, yet the orientation of duplication is tandem in the most cases. To understand the nature of PLP1 duplication, we examined the precise sequences of the duplication breakpoints of three newly identified PMD patients at nucleotide levels. The extent of duplication was examined using various PAC probes along the Xq22 region by interphase FISH. We mapped approximate location of the centromeric and/or telomeric ends of the duplicated segments by quantitative multiplex PCR using sequence-tagged site primers. After detecting the rearranged restriction fragments by Southern blot hybridization, junction fragments were amplified by either oligocassette-mediated PCR or inverse-PCR. Sequence analysis revealed that the duplicated segments, arranged in a head to tail manner, were estimated to be 245 kb, 285 kb and 565 kb in length. The telomeric ends resided in different repetitive elements, L1PA7, AluSp, and L1ME3B, and one of them was located in an X-chromosome specific low-copy repeat (LCR) rich region. There was no homology between the sequences around the centromeric and the telomeric ends except for three to five nucleotides of AT stretch in all three cases. Therefore, it is likely that non-homologous end joining causes PLP1 duplications in these cases.

**Cytoplasmic polyglutamine aggregates are associated with lysosomal proteases.** *K. Ishikawa<sup>1</sup>, T. Makifuchi<sup>2</sup>, H. Fujigasaki<sup>1</sup>, M. Li<sup>1</sup>, S. Toru<sup>1</sup>, T. Kato<sup>3</sup>, K. Nagashima<sup>4</sup>, N. Ohkoshi<sup>5</sup>, E. Kominami<sup>6</sup>, Y. Komatsuzaki<sup>7</sup>, H. Mizusawa<sup>1</sup>.*  
1) Dept Neurology, Tokyo Medical & Dental Univ, Tokyo, Japan; 2) Dept Laboratory Medicien, National Saigata Hospital, Niigata, Japan; 3) 3rd Dept Internal Medicine, Yamagata Univ, Yamagata, Japan; 4) Dept Molecular & Cellular Pathology, Hokkaido Univ, Sapporo, Japan; 5) Dept Neurology, Inst Clinical Medicine, Univ Tsukuba, Ibaraki, Japan; 6) Dept Biochemistry, Juntendo Univ, Tokyo, Japan; 7) Dept Internal Medicine, Hatsuishi Hospital, Chiba, Japan.

Polyglutamine diseases are a group of dominantly-inherited, progressive neurodegenerative diseases caused by expansions of CAG repeat that encode polyglutamine in causative genes. Mutant proteins form aggregations both in nucleus and cytoplasm of neurons, usually undergo degeneration. Although there is an ample evidence that nuclear aggregations of mutant protein is important for pathogenic mechanism of many polyglutamine diseases, nuclear aggregations are very few compared to cytoplasmic aggregations in some polyglutamine diseases, such as SCA2 and SCA6. This might imply that cytoplasmic aggregations could have pathogenic role different from nuclear aggregation. As the first step to elucidate the role of cytoplasmic aggregations, we studied human brains affected with either three polyglutamine diseases, Machado-Joseph disease (MJD; n=5), dentatorubral-pallidoluysian atrophy (DRPLA; n=2), SCA6 (n=5), and analyzed the subcellular location of cytoplasmic aggregates immunohistochemically. The polyglutamine aggregates were detected with anti-expanded polyglutamine antibody, 1C2, and the Golgi apparatus and lysosomal protease were detected with G58K (Sigma) and anti-cathepsin-D antibody, respectively. The cytoplasmic aggregates were more often seen in MJD and SCA6 brains compared to DRPLA. Cytoplasmic polyglutamine aggregates were colocalized with cathepsin-D, but not obviously with G58K. Cytoplasmic aggregates were not obviously labelled with anti-ubiquitin antibody. The present observation may indicate that cytoplasmic aggregates are formed in lysosomes. This process may be a common step in formation of aggregation in polyglutamine diseases.

**Linkage analysis in a large Israeli Bedouin kindred with a unique form of a congenital contractural syndrome.** *G. Narkis*<sup>1,2</sup>, *E. Manor*<sup>2</sup>, *D. Landau*<sup>2</sup>, *K. Elbadour*<sup>2</sup>, *R. Carmi*<sup>2</sup>, *R. Ofir*<sup>1</sup>, *O. Birk*<sup>1,2</sup>. 1) Department of Molecular Genetics, Ben-Gurion University of the Negev, Beer-Sheva, Israel; 2) Genetics Institute, Soroka Medical Center, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

We have recently described a new lethal form of an autosomal recessive multiple contractural syndrome in a large Israeli Bedouin kindred. The phenotype is lethal in the neonatal period and is unique in the presence of a markedly distended urinary bladder. In the present study we set out to determine the genetic locus harboring the gene defective in this disease. We excluded linkage to two known candidate regions associated with autosomal recessive arthrogyrosis (5q35 related to arthrogyrosis multiplex congenita in a large Israeli-Arab inbred family, and 9q34 in the Finnish type of lethal arthrogyrosis). We then performed Genome-wide linkage analysis using the ABI PRISM Linkage Mapping Set. Analysis of 400 polymorphic markers demonstrated linkage to a 6 cM homozygosity region between markers D12S1604 and D12S83 on chromosome 12q13. The maximum Lod score obtained was 3.5 at a recombination fraction of 0. Based on recombination events, the interval harboring the disease-associated locus was further narrowed to a chromosomal region spanning 6.5 Mb between D12S1618 and D12S1072. The linkage established provides means for molecular prenatal diagnosis of the disease in the affected families. Identification of the specific gene defect within the defined region is underway.

**Loss of Neto1, a component of the NMDA receptor complex, is associated with decreased synaptic plasticity and impaired extinction of memories.** *D. Ng<sup>1</sup>, A. Sertie<sup>1</sup>, R.K. Szilard<sup>1</sup>, G.M. Pitcher<sup>2</sup>, S. Clapcote<sup>3</sup>, L.V. Kalia<sup>2</sup>, M.W. Salter<sup>2</sup>, J.C. Roder<sup>3</sup>, M.A. Cortez<sup>2</sup>, R.R. McInnes<sup>1</sup>.* 1) Prog in Devel Biol and; 2) Brain & Behav, Hosp for Sick Children and; 3) Lunenfeld Res Instit, Toronto ON, Canada.

Neto1 & Neto2 are neuronal proteins with two CUB domains and an LDLa domain located extracellularly, and a cytoplasmic tail. Mouse Neto1 & Neto2 have overlapping embryonic and adult expression, being most abundantly expressed in the cerebral cortex and hippocampus, which is essential for memory. The brain of Neto1<sup>-/-</sup> mice is grossly & histologically normal but the mice have myoclonic seizures & defects in some axon projections. To define the functions of Neto1, we first sought its cytoplasmic binding partners. Since Neto1 localizes to the postsynaptic density (PSD) of synapses, and has a C-terminal PDZ binding ligand, we predicted that Neto1 binds to PSD-95, a PSD scaffolding protein. We confirmed this prediction by 2-hybrid assays and co-IPs from crude synaptosomal fractions. Since the NMDA receptor is also assembled by PSD-95, we hypothesized that Neto1 associates with this receptor, which is critical for activity-dependent synaptic plasticity in the hippocampus. Neto1 and components of the NMDA receptor co-IP, and Neto1 interacts directly with the NR1 subunit of the receptor, as shown by 2-hybrid and co-IP studies. To determine whether NMDA receptor function is disrupted in Neto1<sup>-/-</sup> mice, we measured LTP, a test of synaptic strength, in hippocampal slices, and identified a decrease in LTP amplitude. To test whether the LTP defect impairs memory, we examined Neto1 mutants in the Morris water maze. Neto1<sup>+/-</sup> and Neto1<sup>-/-</sup> mice acquired new memories as well as Neto1<sup>+/+</sup> mice, but had difficulty extinguishing memories, a phenomenon seen in some patients with psychiatric disorders. We conclude that 1) Neto1 is a component of the NMDA receptor complex and directly binds NR1; 2) Neto1<sup>-/-</sup> mice have defects in synaptic plasticity; 3) Neto1 is required for memory extinction; 4) human NETO1 is a candidate for CNS diseases, particularly the bipolar disorder locus at 18q22, epilepsy, and other disorders affecting the synapse.

Program Nr: 2233 from 2003 ASHG Annual Meeting

**Pro-survival effects of wild-type huntingtin *in vivo*.** *B.R. Leavitt, D. Rogers, J. vanRaamsdonk, C.J. Opina, E. Chan, C.L. Wellington, M.R. Hayden.* CMMT, Univ British Columbia, Vancouver, BC, Canada.

A pro-survival function for huntingtin was first postulated by Nasir and colleagues in 1995, based on the presence of neurodegeneration in mice heterozygous for deletion of the Hdh gene. We developed cell culture based assays in which we model the pro-survival effects of huntingtin in several types of cell death. These assays allow us to elucidate the cellular mechanisms by which wild-type huntingtin exerts its pro-survival effects. In addition to our *in vitro* studies we have found that over-expression of wild-type huntingtin confers dramatic protection against caspase activation and apoptotic neurodegeneration in transgenic mice. Following kainic acid-induced seizures, YAC transgenic mice expressing 2-3 times the endogenous levels of wild-type htt averaged approximately 50-fold less degenerating hippocampal neurons than control animals (81 vs. 3708,  $p < 0.006$ ). Significantly less hippocampal caspase activation was also evident by DEVDase assay in transgenic mice compared to wild-type mice (14.5 vs. 20.8,  $P = 0.02$ ). Transgenic mice that over-express the mutant form of huntingtin are not protected. Activation of caspases and cleavage of full-length huntingtin leads to decreased neuronal huntingtin levels and apoptotic death of hippocampal neurons following kainic acid-induced seizures. This neuronal cell death is completely blocked by ZVAD-fmk an inhibitor of caspases. These data provide *in vivo* evidence that huntingtin levels can modulate neuronal sensitivity to excitotoxic neurodegeneration. We propose that reduced levels of wild-type huntingtin and the resultant loss of pro-survival huntingtin function may contribute to the increased vulnerability of striatal neurons to excitotoxic degeneration in Huntingtons disease.

**Genotype-phenotype correlation of hereditary sensory neuropathy type 4: Extension of the phenotype. C.**

*Oddoux*<sup>1</sup>, *C. Maayan*<sup>3</sup>, *R. Cilio*<sup>4</sup>, *F. Axelrod*<sup>2</sup>. 1) Div Human Genetics, New York Univ Sch Medicine, New York, NY; 2) Pediatrics, New York Univ Sch Medicine, New York, NY; 3) Hadassah Hospital, Mt Scopus, Jerusalem, Israel; 4) Ospedale Pediatrico Bambino Gesù, Piazza S. Onofrio, Rome, Italy.

Hereditary sensory and autonomic neuropathies (HSAN) are a group of genetic disorders with overlapping characteristics. HSAN types 2, 3, 4, and 5 are inherited as autosomal recessive traits. Mutations in the *IKAP* gene have been associated with HSAN type 3 (also known as familial dysautonomia) and mutations in the *NTRK1* gene (nerve growth factor receptor) have been associated with HSAN type 4 (also known as CIPA). The genes responsible for HSAN types 2 and 5 have yet to be discovered.

The overlap in phenotypic characteristics of these disorders can make their delineation difficult. HSAN 4 is distinguished from the other disorders in the group by a decreased ability to sweat. Some patients classified as having HSAN 4 by these criteria have a milder phenotype retaining partial pain perception and partial ability to sweat. In an effort to correlate diagnostic criteria used to delineate HSAN 4 with genetic causative agents, we sequenced the *NTRK1* gene in a series of patients characterized with HSAN type 4 including some that had what appeared to be a milder phenotype. All seventeen exons and intron-exon boundaries were sequenced. Not all patients classified as having HSAN4 had detectable mutations. Mutations were detected in some patients displaying the milder phenotype and some patients with the classical phenotype had no detectable mutations. Our results are consistent with a broader phenotypic spectrum for HSAN4 and genetic etiologies for this disorder other than *NTRK1* coding region mutations.

**Full-length DRPLA protein suppresses CREB- and p53- dependent transcriptional activation.** *T. Shimohata<sup>1</sup>, K. Ogura<sup>1</sup>, S. Naruse<sup>1</sup>, S. Igarashi<sup>2</sup>, T. Sato<sup>3</sup>, O. Onodera<sup>2</sup>, M. Nishizawa<sup>1</sup>, S. Tsuji<sup>4</sup>.* 1) Department of Neurology, Brain Research Institute, Niigata University; 2) Resource Branch for Brain Disease Research, Center for Bioresource-based Researches, Brain Research Institute, Niigata University, Niigata, Japan; 3) Animal Resources Branch, Center for Bioresource-based Researches, Brain Research Institute, Niigata University, Niigata, Japan; 4) Department of Neurology, University of Tokyo, Graduate School of Medicine, Tokyo, Japan.

At least nine neurodegenerative diseases, including dentatorubral pallidoluysian atrophy (DRPLA), have been known to be caused by expanded CAG repeats encoding polyglutamine (polyQ) stretches. Recently, it has been demonstrated that interference with CREB-dependent transcription due to the binding of expanded polyQ stretches with transcriptional co-activators, such as TATA-binding protein associated factor (TAFII130) or CREB-binding protein (CBP), is involved in the pathogenetic mechanisms underlying neurodegeneration. It remains, however, unclear whether full-length DRPLA proteins have physiological functions on transcriptional regulation. To investigate the effects of DRPLA proteins on transcriptional regulation, we performed a reporter assay (dual luciferase assay) using cultured cells expressing full-length or truncated DRPLA proteins containing 19 or 82 glutamines, and found that full-length DRPLA proteins suppressed CREB-dependent transcriptional activation. There are no apparent differences of the suppression levels between the cells expressing DRPLA proteins containing 19 or 82 glutamines. We also found that p53-dependent transcriptional activation was suppressed by full-length DRPLA proteins, while AP1- or NF $\kappa$ B-dependent transcriptional activation was not suppressed. Taken together, these results raise the possibility that physiological function of DRPLA proteins is the transcriptional regulation that depends on CREB or p53.

**Autosomal dominant cerebellar ataxia with sensory neuropathy maps to the spinocerebellar ataxia 25 (SCA25) locus on chromosome 2p15-p21.** *G. Stevanin<sup>1</sup>, N. Bouslam<sup>1</sup>, E. Broussolle<sup>3</sup>, L. Ravaux<sup>1</sup>, A. Boland<sup>4</sup>, A. Durr<sup>1,2</sup>, A. Brice<sup>1,2</sup>.* 1) INSERM U289-NEB, Institut Federatif des Neurosciences, Paris, France; 2) Departement de Genetique, Cytogenetique et Embryologie, AP-HP, Groupe hospitalier Pitie-Salpetriere, Paris, France; 3) Service de Neurologie, Hopital Neuro-chirurgical Pierre Wertheimer, Lyon, France; 4) Centre National de Genotypage, Evry, France.

Autosomal dominant cerebellar ataxias constitute one of the most clinically, neuropathologically and genetically heterogeneous group of neurodegenerative disorders. Approximately 50-80% of the families do not have mutations in genes known to be implicated in spinocerebellar ataxias (SCA). Numerous SCA loci have also been mapped, often in single families, but the responsible genes have not been identified yet. This suggests further genetic heterogeneity. We have ascertained 18 individuals from a large French family in which cerebellar ataxia and prominent sensory neuropathy segregated as an dominant trait. Interfamilial variability was great regarding age at onset (17 months to 39 years), severity and the clinical picture, ranging from sensory neuropathy to Friedreichs ataxia-like phenotype. After excluding known SCA mutations, linkage was detected, in a genome wide screen, with markers on chromosome 2p, thereby defining a new locus that we designated SCA25. For confirmation of linkage and fine mapping of the genetic interval, 18 additional markers were tested. A maximum two-point lod score of 3.15 was obtained at D2S2378. Haplotype analysis defined a critical 12.6-cM-region between D2S22174 and D2S2736. This interval contains several genes that could be responsible for the disease. One of these genes, CRIPT, encodes a post-synaptic protein. No mutations were found in its entire coding region, excluding it as a candidate. Furthermore, CAG repeat expansions are often involved in SCA pathogenesis, but no pathological expansions were found at the protein or at the DNA level using the 1C2 antibody and the Repeat Expansion Detection method, respectively. The gene responsible for SCA25 remains to be determined.

**Mutation of the Paired-like Homeobox 2B gene (PHOX2B) in congenital central hypoventilation syndrome**

**(CCHS): vertical transmission and phenotype/genotype correlations.** *D. Trochet<sup>1</sup>, K. Waters<sup>2</sup>, H. Trang<sup>3</sup>, B. Laudier<sup>1</sup>, L. de Pontual<sup>1</sup>, A. Munnich<sup>1</sup>, C. Gaultier<sup>3</sup>, S. Lyonnet<sup>1</sup>, J. Amiel<sup>1</sup>.* 1) INSERM U393, Hopital Necker, Paris, France; 2) Respiratory Support Service, The Children's Hospital at Wesmead, Sydney, Australia; 3) Service de Physiologie, Hopital Robert Debre, Paris, France.

CCHS (Ondine's curse, MIM209880) is a life-threatening disorder involving an impaired ventilatory response to hypercarbia and hypoxaemia. This core phenotype is associated with lower penetrance anomalies of the autonomic nervous system (ANS) including Hirschsprung disease (Haddad syndrome, MIM142623) and tumours of the sympathetic nervous system. Considering that the development of ANS reflex circuits is Phox2b-dependent in mice, we showed that most CCHS cases are ascribed to mutations of its human ortholog PHOX2B\*. We further screened the PHOX2B gene in a series of 43 patients including 30 isolated CCHS, 13 Haddad syndrome, and 3 patients with CCHS and neuroblastoma or ganglioneuroma. Heterozygous de novo PHOX2B mutations were found in 24/30 sporadic CCHS and 8/13 Haddad syndrome cases respectively, suggesting that isolated CCHS and Haddad syndrome are allelic conditions at the PHOX2B locus. Most PHOX2B mutations consisted in 5-9 alanine expansions within a 20 alanine tract likely to result from non-homologous recombination (28/32). The four remaining mutations were frameshift mutations resulting in an elongated (3/4) or shortened putative protein (1/4) with a preserved homeodomain. Although no genotype/phenotype correlation can be established as far as the length of the polyalanine expansion is concerned, frameshift mutations may predispose to tumors, as opposed to polyalanine expansions. Finally, we identified a 5-alanine expansion of the PHOX2B gene in a familial case where the affected father transmitted a stable mutant allele to his affected daughter further supporting an autosomal dominant inheritance with de novo PHOX2B mutations at the first generation. Combined with our expression study, our data support the crucial role of PHOX2B in the patterning of the autonomous ventilation system and ANS in human.\*Nat Genet 2003;33:459-61.

**An intragenic modifier of hereditary spastic paraplegia due to spastin gene mutation suggests a role for cyclin-dependent kinase 5 in HSP pathogenesis.** *I.K. Svenson<sup>1</sup>, M.T. Kloos<sup>1</sup>, P.C. Gaskell<sup>1</sup>, M.A. Nance<sup>2</sup>, J.Y. Garbern<sup>3</sup>, M.A. Pericak-Vance<sup>1</sup>, A.E. Ashley-Koch<sup>1</sup>, D.A. Marchuk<sup>1</sup>.* 1) Duke University Medical Center, Durham, NC; 2) Park Nicollet Clinic, Minneapolis, MN; 3) Wayne State University School of Medicine, Detroit, MI.

Hereditary spastic paraplegia (HSP) is a genetically heterogeneous neurodegenerative disease characterized by wide variability in phenotypic expression, both within and among families. The most common cause of autosomal dominant HSP is mutation of the gene encoding spastin, a protein of unknown function. Almost all reported missense mutations in spastin are located in the C-terminal half of the protein, which contains the highly conserved AAA domain. The sole exception is a serine to leucine substitution at amino acid residue 44. This mutation was found previously in only one individual, in whom it occurred homozygously, and it was speculated to be a rare, recessively acting mutation. We have now identified S44L as a North American population polymorphism. In addition, we identified a family in which S44L segregates independently of D470V, a missense mutation in the AAA domain of spastin. S44L was associated with a striking decrease in age at onset and increase in severity of symptoms in the presence of the D470V mutation in this family. S44L is located in the poorly characterized amino-terminal half of the protein. Using a bioinformatics approach, we found that this highly conserved serine residue is predicted to be a site of phosphorylation by the proline-dependent serine/threonine cyclin-dependent kinase 5 (Cdk5). Cdk5 is abundant in the brain, where it is associated with complexes of cytoskeletal proteins including tubulins, consistent with a previously reported role for spastin in regulating microtubule dynamics. In addition, Cdk5 plays a major role in the molecular path leading to neurodegeneration. Thus, our identification of S44L as a modifier of the HSP phenotype has led to new insights into the biochemical pathways involved in the neurodegeneration in HSP due to spastin mutation.

**FMR1 Testing in Subjects with Undiagnosed Ataxia Disorders.** *K.J. Steenblock<sup>1</sup>, S.A. Adams<sup>1</sup>, S.N. Thibodeau<sup>1</sup>, N.M. Lindor<sup>2</sup>.* 1) Division of Laboratory Genetics, Mayo Clinic, Rochester, MN; 2) Department of Medical Genetics, Mayo Clinic, Rochester, MN.

Fragile X Ataxia Syndrome (FXAS) is a newly defined tremor and ataxia syndrome in male FMR1 premutation carriers. Symptoms of this disorder include progressive intention tremor, ataxia, cognitive decline, and characteristic MRI findings. Elevated FMR1 mRNA levels are hypothesized to cause buildup of inclusions, resulting in the distinct MRI findings and phenotype. Initial studies reported these features in males with known family histories of Fragile X syndrome. Recent studies identified gray zone FMR1 alleles (40-60 CGG repeats) in a subset of patients with multiple system atrophy (MSA). To date, the proportion of male ataxia patients with either etiology is unknown. In this study, PCR analysis was performed on 286 males over age 40 who were tested for Dentatorubral-Pallidoluysian Atrophy (DRPLA) (N=3), Spinocerebellar Ataxia (SCA) (1,2 and 3 or 1,2,3,6 and 7) (N=269), or both (N=14). Clinical information was obtained on each of the 286 patients. Features most commonly identified in subjects were gait ataxia, dementia, tremor, tingling and/or numbness, spasticity, muscle weakness, and neuropathy. Of the 286 subjects tested, one premutation (100 CGG repeats) and 16 "gray zone" alleles were identified. Although the premutation rate in this study is roughly equivalent to that of the general population, the results yield pertinent clinical applications. Of the subjects studied, 54% had only one of the three cardinal FXAS features, 21% had two of the three, and 4% had all three. Gait ataxia associated with clinical features not typically described in FXAS was found in 46% of subjects. These findings suggest that gait ataxia in the absence of tremor and dementia is less likely to result in the identification of an FMR1 premutation than if all three features were present. It is possible, however, that a higher frequency of FMR1 premutations would be identified in a more select population. Further research is necessary to define specific clinical features of this syndrome to allow for more effective use of FMR1 testing.

**Huntington's disease-like 2 (HDL2): A CTG repeat expansion in Junctophilin-3 leads to a loss of function.** *D. Rudnicki<sup>1</sup>, S.E. Holmes<sup>1</sup>, E. O'Hearn<sup>2,3</sup>, J. Troncoso<sup>4</sup>, J. Hwang<sup>1</sup>, O. Pletnikova<sup>4</sup>, C.A. Ross<sup>1,2,3,5</sup>, H. Takeshima<sup>6</sup>, T.H. Moran<sup>1</sup>, R.L. Margolis<sup>1,2,5</sup>.* 1) Psychiatry; 2) Neurology; 3) Neuroscience; 4) Pathology; 5) Program in Cellular and Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 6) Department of Biochemistry, Tohoku University, Japan.

HDL2 is a devastating neurological disorder with clinical and pathological findings essentially indistinguishable from Huntington's disease (HD). The causative mutation is a CAG/CTG expansion in the CTG orientation within a variably spliced exon of junctophilin-3 (JPH3). We hypothesize that the expansion results in a loss of JPH3 function through interference with transcription or splicing. To test this hypothesis, we performed RT-PCR across the JPH3 repeat in frozen HDL2 brain tissue. The normal repeat, but not the expanded repeat, was amplified in each case, though both repeats could be amplified from each case in genomic DNA. RT-PCR across the full-length JPH3 transcript suggested the presence of abnormal splice variants in patient genomic, consistent with expansion-induced alterations in JPH3 splicing. To further test the loss-of-function hypothesis, we investigated knockout mice hemizygous for the junctophilin-3 gene. These mice were motorically impaired, as measured by the rotarod, and gradually became hypoactive. Preliminary immunohistochemical evidence suggests that these mice have neuronal intranuclear inclusions, similar to those seen in HDL2 brain, stained by anti-polyglutamine and anti-ubiquitin antibodies. In combination, this evidence suggests that the JPH3 repeat expansion in HDL2 results in a decrease in junctophilin-3 expression. How this loss of expression leads to neuronal inclusions and neurotoxicity remains to be determined. Funding sources: This work was supported by a Lieberman Award from the Hereditary Disease Foundation.

**Astrocytic expression of the cerebral cavernous malformations type 1 gene, *KRIT1*, and its interaction partner *ICAP1* suggests a role in formation or maintenance of the blood-brain barrier.** *J.S. Zawistowski*<sup>1,3</sup>, *N.W. Plummer*<sup>1,3</sup>, *D.D. Chang*<sup>2</sup>, *D.A. Marchuk*<sup>1</sup>. 1) Dept of Molec. Genetics and Microbiology, Duke University, Durham, NC; 2) Dept of Medicine, UCLA School of Medicine, Los Angeles, CA; 3) contributed equally.

Cerebral cavernous malformations (CCM) are vascular lesions of the central nervous system consisting of clusters of greatly enlarged, thin-walled blood vessels without intervening brain parenchyma. Hemorrhaging of the lesions can result in migraines, seizures, or lethal stroke. Three forms of autosomal dominant cerebral cavernous malformations have been mapped, and CCM1 is caused by loss-of-function mutations in a novel gene termed *KRIT1*. The *KRIT1* protein contains ankryin repeats and a FERM domain in the carboxyl half of the protein, but the amino half of the protein is unique. It remains unclear how loss of *KRIT1* contributes to CCM pathogenesis, and even if the pathology is intrinsic to vascular endothelial cells. *In-situ* analyses have suggested that *KRIT1* is expressed in neurons, whereas an antibody raised against a C-terminal peptide has shown *KRIT1* expression in cultured endothelial cells. In order to resolve these issues, we have generated an antibody to a GST-fusion protein consisting of the unique N-terminal 272 amino acids of *KRIT1* which harbor the motif critical for binding to the  $\alpha_1$  integrin-associated protein *ICAP1*. We have used our *KRIT1* antibody and a previously described *ICAP1* antibody to investigate the expression pattern of both genes.

Our analyses demonstrate that *Krit1* and *Icap1* are not expressed in neurons, nor in the vascular endothelium of adult mouse brain. Instead, both proteins show strong expression in astrocytes. Since interaction between astrocytes and vascular endothelial cells is known to be required for formation of the blood-brain barrier (BBB), this expression pattern suggests a role for *KRIT1* in formation or maintenance of the BBB. These data are also consistent with previously reported observations that endothelial cell tight junctions are defective in cavernous malformations, and suggest that the interaction between astrocytes and endothelial cells depends on  $\alpha_1$  integrin.

**Molecular analysis of NAIP gene in Iranian Spinal Muscular Atrophy families.** *I. Salahshouri<sup>1</sup>, Y. Safagati<sup>1,2</sup>, Z. Golkar<sup>2</sup>, H. Najmabadi<sup>1,2</sup>.* 1) The Social Welfare and Rehabilitation Sciences University, Evin, Tehran, Iran; 2) 2 Kariminejad/Najmabadi genetics and pathology center.

**Abstract:** Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by degeneration of anterior horn cells in the spinal cord leading to progressive muscular weakness and atrophy. The three candidate genes for SMA were mapped to the 5q13 region. SMA-determining genes including SMN (survival motor neuron), (the major gene in SMA phenotype), NAIP (neuronal apoptosis inhibitory protein) and BTF2P44, all of which are located on chromosome 5q13 in two highly homologous copies (telomeric and centeromeric) within the SMA region. The objective of this study was to investigate the NAIP gene deletion in SMA patients and families. NAIP gene deletion analyzed in 34 SMA families as mentioned above, in which exon 7 of SMNt gene before confirmed and in 75% exon 7 of SMNt gene was deleted. We found 60% (12/20) NAIP gene deletion in SMA I-III (77%; SMA-I, 29% SMA-II or III), 6.6% (2/30) carrier parents and none of the normal parents. The NAIP gene deletion in SMA-I was higher other than SMA-types. In this study consanguinity rate was 65% (22/34). **Key words:** Spinal Muscular Atrophy; the SMN gene; the NAIP gene; P44 gene.

**Molecular genetic analysis in Moebius syndrome.** *B. van der Zwaag*<sup>1</sup>, *H.T.F.M. Verzijl*<sup>1</sup>, *D. Beltran Valero de Bernabe*<sup>2</sup>, *J. Veltman*<sup>2</sup>, *H.G. Brunner*<sup>2</sup>, *G.W. Padberg*<sup>1</sup>, *H. van Bokhoven*<sup>2</sup>. 1) Neurology, UMC Nijmegen, Nijmegen, Gelderland, The Netherlands; 2) Human Genetics, UMC Nijmegen, Nijmegen, Gelderland, The Netherlands.

Moebius syndrome (MBS) is a rare autosomal dominant disorder characterized by paralysis of the 7<sup>th</sup> cranial nerve (facialis). Additional clinical features in MBS are dysfunction of other cranial nerves, limb- and musculoskeletal malformations, and mental retardation. Several loci for MBS have been uncovered by linkage studies and cytogenetic analysis. We initiated a positional candidate gene analysis for MBS2 (3q21-q22) and MBS3 (10q21-q22), the loci identified in our lab in two large non-related Dutch MBS families. Several positional candidate genes were excluded, including *EGR2*, *GATA2*, *PGT*, and *SOX14*. Recently, we identified a new candidate gene: *PLEXIN-D1*. RNA *in situ* hybridization during mouse embryogenesis revealed that *PLEXIN-D1* is expressed in regions involved in MBS (brain and cranial ganglia), and in developing blood vessels. Mutation analysis in the MBS2 family and in a cohort of over 40 isolated MBS patients identified 19 nucleotide changes in the coding sequence and splice sites of the gene. However, all of these were present in the normal population and should be considered polymorphisms. RT-PCR analysis showed no aberrations in splicing, and the co-segregating nucleotide changes were heterozygously present in the RNA, demonstrating promoter activity for both alleles. Therefore *PLEXIN-D1* can be excluded as the causative gene for MBS2. To select new candidate genes for MBS2, we are currently performing large scale RNA *in situ* analysis for 22 genes from the critical region. Transcripts showing expression in MBS affected tissues are considered new candidate genes. Selection of candidate genes will be followed by mutation analysis, to identify the causative mutation for MBS2. In addition, DNA samples from over 40 isolated MBS patients are being hybridized to a newly developed genomic microarray to detect possible chromosomal aberrations (deletions or duplications) in MBS patients. The array contains over 3600 BACs, resulting in a 5 to 10 times higher resolution than routine karyotyping.

**MUTATION DETECTION OF SOME NEUROMUSCULAR DISEASES IN MOLDOVA.** *V.C. Sacara.* Medical genetics Centre, Scientific Research Institute , Kishinau, Moldova.

Progress in molecular genetics has greatly improved our understanding of the molecular basis of many inherited neurological diseases and to provide practical help for the clinical neurologist to make appropriate use of the possibilities of molecular diagnosis of neurologic disorders in Moldova. In our data base we have 120 families with high genetic risk of Duchenne/Becker muscular dystrophy (DMD), 30 families with Spinal muscular atrophy (SMA), 15 families with Charcot-Marie-Tooth disease(CMT1A). Most of these families are Moldavian by origin. DNA analysis of the DMD families consists of detects deletions in 13 different exons of dystrophin gene and pERT87-8/TaqI, pERT87-15/BamH1 and 16 intron/TaqI polymorphisms. About 76 % of probands were proved to be carriers of dystrophin gene deletion by MPCR. The algorithm of MDD molecular researches allows to define informative in 93% cases. Molecular analysis was efficiently applied to 11 fetuses. Five male fetuses were no affected, two male fetuses were affected, in one case the risk remained 50% and in 3 cases it were heterozygous carriers. Molecular studies at the SMNI - homozygous deletions of exons 7 and 8 were found in 21 patients (91%). In 3 patients was observed only exon 8 deletions. The feasibility of indirect diagnosis was evaluated using microsatellite polymorphic locus D5S557. Four alleles (148bp, 160bp, 166bp, 172bp) were exposed. The most frequent allele in control group had 160 bp length. This allele accounted 52% of all alleles. The observed heterozygosity rate was 0,54. A total 23 DNA samples from patients and 40 relatives, were analyzed. 74% of families were informative and allele marked mutant chromosome was detected. In the most case it was allele 166 bp length with frequency 0,48. In 5 cases we performed prenatal diagnosis SMA. In 3 out of 4 fetuses we detected heterozygous deletion of the exons. We use of STRs for diagnosis of CMT1A duplication. Two markers (17dup5 and 17dup4) were amplified by PCR using standard conditions. We found duplication in 8 CMT1A families. DNA-analysis supplements considerably clinic-laboratorial methods of diseases investigation, permits to use for effective and rapid carrier detection and prenatal diagnosis in at-risk families.

**Analyses of biochemical function of aprataxin in DNA single-strand break repair.** *T. Takahashi*<sup>1</sup>, *S. Igarashi*<sup>1,2</sup>, *H. Date*<sup>1</sup>, *O. Onodera*<sup>1,2</sup>, *M. Nishizawa*<sup>1</sup>, *S. Tsuji*<sup>3</sup>. 1) Department of Neurology, Brain Research Institute, Niigata University, Niigata, Japan; 2) Department of Molecular Neuroscience, Brain Research Institute, Niigata University, Niigata, Japan; 3) Department of Neurology, The University of Tokyo, Tokyo, Japan.

Aprataxin (APTX) has been identified as the causative gene for Early-onset ataxia with ocular motor apraxia and hypoalbuminemia (EAOH). The physiological functions of aprataxin, however, remains to be elucidated. APTX has two splicing variants, long form(LAPTX) and short form. LAPTX has a homology to polynucleotide kinase-3' phosphatase (PNKP) and interacts with XRCC1, which is required for single-strand DNA break repair(SSBR), in a yeast two-hybrid study. To investigate the functions of the aprataxin in SSBR, we conducted in vitro reconstituted SSBR assay using oligonucleotide duplex with a 1 bp gap and three recombinant SSBR-related proteins (DNA polymerase(Pol), DNA ligase III (Lig3) and T4 polynucleotide kinase(T4 PNK). The short fragment of the oligonucleotides was end-labelled with fluorescein isothiocyanate, and the length of ligated oligonucleotide products was analyzed by an automated DNA sequencer. In this experiment, reconstituted SSBRs were completed by recombinant lig3, Pol and T4 PNK ,however, ligation products were undetectable by using LAPTX substituted for T4 PNK. This data suggested that LAPTX does not have any polynucleotide kinase activity in spite of its homology. To elucidate the physiological roles of aprataxin in SSBR ,further investigations on other SSBR systems will be needed.

**Autosomal dominant hypoceruloplasminemia associated with extrapyramidal signs due to a novel heterozygous 4-bp deletion mutation in the Ceruloplasmin gene.** *Y. Watanabe<sup>1</sup>, Y. Yamashita<sup>1</sup>, T. Yamamoto<sup>2</sup>, E. Nanba<sup>3</sup>, M. Nakashima<sup>1</sup>, I. Yoshida<sup>1</sup>, T. Matsuishi<sup>1</sup>, M. Yoshino<sup>1</sup>.* 1) Department of Pediatrics, Kurume University, Fukuoka, Japan; 2) Division of medical genetics, Kanagawa children's medical center, Kanagawa, Japan; 3) Division of Functional Genomics, Research Institute of Bioscience and Technology, Tottori University, Tottori, Japan.

We identified a novel 4-bp deletion in exon 14 at position 2520 of the Ceruloplasmin(CP) gene in a Japanese family with hypoceruloplasminemia, resulting in a premature termination at amino acid 892. The proband was a 12-year-old boy who was presented with a history of refusing attending school because of his motor clumsiness and tremor which had been noticed since age four years. He had mild mental retardation and extrapyramidal signs including dysarthria and cogwheel rigidity. Serum ceruloplasmin and copper were 10 mg/dl (normal 22-44) and 41 g/dl (normal 80-130), respectively. Wilson disease was excluded by normal urine copper excretion and normal copper content in the liver tissue. Hypoceruloplasminemia and heterozygous mutation of the 4-bp deletion were identified in four individuals in this family (father: CP 13, Cu 52; younger brother: CP17, Cu 65; paternal grandmother: CP 17, Cu 56). Abnormal extrapyramidal signs as well as abnormal brain MRI findings with high signal intensity in globus pallidus were identified only in the proband and his father. The other two relatives were found asymptomatic. We report a familial hypoceruloplasminemia, due to a novel heterozygous 4-bp deletion mutation of the CP gene, resulting in abnormal neurological findings in a father and a son. Hypoceruloplasminemia due to the deletion mutation at nucleotide 2520 showed autosomal dominant inheritance with variable expression in neurological symptoms. Although onset of neurological symptoms in hypoceruloplasminemia was believed to be between 3<sup>rd</sup> and 4<sup>th</sup> decades, this familial cases suggest that onset of neurological symptoms can be significantly earlier (as early as age four years) than it was thought.

**Paraplegin mutations are associated to complex I deficiency and increased sensitivity to oxidative stress in Hereditary Spastic Paraplegia.** *L. Silvestri<sup>1</sup>, L. Cassina<sup>1</sup>, L. Atorino<sup>1</sup>, M. Koppen<sup>2</sup>, A. Ballabio<sup>3</sup>, R. Marconi<sup>4</sup>, T. Langer<sup>2</sup>, G. Casari<sup>1</sup>.* 1) Neuroscience, San Raffaele Scientific Instit, Milan, MI, Italy; 2) Institute for Genetics and Center for Molecular Medicine, University of Cologne, Cologne, Germany; 3) Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy; 4) Division of Neurology, Ospedale Misericordia, Grosseto, Italy.

Mutations in paraplegin, a putative mitochondrial metallopeptidase of the AAA family, cause an autosomal recessive form of hereditary spastic paraplegia (HSP). We analyze the function of paraplegin at the cellular level and characterize the phenotypic defects of HSP patients cells lacking this protein. Paraplegin and the homologous protein AFG3L2 are integral proteins of the inner mitochondrial membrane; by coimmunoprecipitation and FPLC experiments, we demonstrate that paraplegin interacts with AFG3L2 to form a high molecular weight complex of about 900 kDa, which is aberrant in HSP fibroblasts. The loss of this complex causes a reduced complex I activity in mitochondria and an increased sensitivity to oxidant stress, which can be both rescued by exogenous expression of wild type paraplegin. The impaired complex I activity correlate to a reduced amount of complex I in HSP cells, we therefore conclude that the paraplegin/AFG3L2 complex is required for the efficient assembly of complex I. Furthermore, complementation studies in yeast demonstrate functional conservation of the human paraplegin/AFG3L2 complex with the yeast m-AAA protease and also assign proteolytic activity to this structure. Taken together, our findings shed new light on the molecular pathogenesis of HSP. We show how paraplegin mutations suppress the human m-AAA protease formation and generate complex I deficiency in HSP cells, which may lead to additional production of ROS causing oxidative stress in the particular subset of neurons affected by HSP. Therefore, the initial pathogenic mechanism is likely to stem from the combined effects of misfolded peptide accumulation and the lack of chaperon activity on complex I assembling.

**Somatic mosaicism in Fanconi anemia: Investigation of the dynamics of phenotype correction.** *Y. Akkari, Y. Torimaru, M. Grompe, S.B. Olson.* Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR.

Fanconi anemia (FA) is an autosomal recessive disease characterized by congenital anomalies, bone marrow failure, and an increased risk for neoplasia. Cells from FA patients are hypersensitive to DNA crosslinking agents, such as mitomycin C (MMC), and display genomic instability. Recently, it has been recognized that the genomic instability seen in FA cells may increase the opportunity for correction of the genetic error in a proportion of the patient's blood cells by a phenomenon known as somatic reversion. In order to simulate somatic reversion seen occasionally in FA, tissue samples from several FA patients were mixed in different proportions with like material from sex-unmatched healthy individuals, and treated with and without MMC. Breakage analysis and fluorescent in situ hybridization (FISH) were performed to assess the resulting sensitivity and the proportion of male and female cells in the mixed samples, respectively. Our results suggest that in all blood, marrow, and fibroblast samples treated with MMC, the presence of only 5% healthy cells can decrease the radial percent reading to normal ranges (ie. >20% radials). In contrast to untreated mixed cultures, blood cultures treated with MMC revealed that FA cells were more prone to cell death which is consistent with the selective advantage of the reverted cell population. In addition, breakage analysis of FA cells in the normal cell milieu, as followed by FISH for female versus male cells, revealed no evidence for intercellular correction for MMC hypersensitivity. These findings have implications for the treatment of FA in general, as well as for the longterm survival of FA patients following a somatic reversion event.

**Identification of the Gene Causing CM-AVM, a Heretofore Undescribed Clinical Entity, Characterized by Defects in Capillary Morphogenesis, Arterial/Venous Identity and Control of Local Tissue Growth.** *I. Eerola<sup>1</sup>, L.M. Boon<sup>1, 2</sup>, J.B. Mulliken<sup>3</sup>, P.E. Burrows<sup>4</sup>, R. Vanwijck<sup>2</sup>, M. Vikkula<sup>1</sup>.* 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology & Université catholique de Louvain, Brussels, Belgium; 2) Centre for Vascular Anomalies, Division of Plastic Surgery, Cliniques universitaires St-Luc, Université catholique de Louvain, Brussels, Belgium; 3) Vascular Anomalies Center, Division of Plastic Surgery, Children's Hospital, Harvard Medical School, Boston, MA 02115, USA; 4) Vascular Anomalies Center, Division of Interventional Radiology, Children's Hospital, Harvard Medical School, Boston, MA 02115, USA.

Defects in cutaneous vascular development manifest as vascular malformations that vary in size, location, blood flow, and clinical severity. Capillary malformation (CM), (port-wine stain), (OMIM#163000) is the most common one, occurring in 0.3% of newborns. Arteriovenous malformation (AVM) and arteriovenous fistula (AVF) are fast-flow vascular anomalies that arise in skin, muscle, bone, internal organs and brain, and can cause life-threatening complications, such as bleeding, congestive heart failure, or neurologic consequences. Multiple micro-AVFs co-occur with cutaneous stain, and soft tissue and skeletal hypertrophy of the affected limb in Parkes Weber syndrome. Using linkage analysis and positional candidate gene screening, we have identified a gene, at the CMC1 locus on chromosome 5q, mutations of which can cause CM, AVM, AVF and Parkes Weber syndrome. This data delineates a new phenotypic and genetic entity that we named CM-AVM. The variability in phenotype can be explained by the involvement of the identified mutated gene in signaling of various growth factor receptors that control proliferation, migration, and survival of several cell types, including vascular endothelial cells. (vikkula@bcm.ucl.ac.be) (<http://www.icp.ucl.ac.be/vikkula>).

**INCIDENCE OF TRANSTHYRETIN VAL122ILE AMYLOID MUTATION IN AFRICAN-AMERICANS BORN IN INDIANAPOLIS, INDIANA, USA.** *M. Benson, M. Yazaki, T. Yamashita, K. Hamidi Asl.* Dept Path & Lab Medicine, Indiana Univ, Indianapolis, IN.

Hereditary transthyretin amyloidosis is associated with 85 different mutations in transthyretin. While the most common type of transthyretin amyloidosis in the world is caused by the TTR Val30Met mutation, one particular TTR mutation (Val122Ile) is found predominantly in Americans of African descent. This mutation causes late-onset restrictive cardiomyopathy (after age 60). In previous reports allele frequencies of 0.014 (148 subjects) and 0.020 (1688 subjects) were found. Those studies, however, used DNA samples obtained for genetic analysis of other diseases in various geographic areas. The present study determines the gene frequency in newborns in a mid-American city (Indianapolis, Indiana). Cord bloods were collected from 1,973 subjects. Val122Ile was detected by PCR-RFLP.

Race	Number Tested	Number Positive	% Positive
African-American	1,000	30	3%
Caucasian	453	2	.004%
Hispanic	490	0	
Other (Asian, etc.)	30	0	

Conclusion: In a population selected only by ethnicity and birth in an urban community the finding of 3% incidence of the TTR Val122Ile mutation associated with late-onset cardiac amyloidosis is higher than previously reported. The amyloidosis caused by this mutation is often clinically mistaken for other types of cardiac disease in the African-American population. Demonstration of the high incidence of this mutation in African-Americans is important for the education of individuals giving care to this population.

**Compound heterozygous mutation of both and subunits of IKs associated with Romano-Ward Syndrome.** *E. Estrella, T. Miller, L.J. You, W. Lanier, M.L. Young, R. Myerburg, N. Bishopric, L. Baumbach.* Univ. Miami Sch. Med., Miami, FL.

Inherited forms of Long QT Syndrome (LQTS) are caused by over 200 mutations in seven genes. Mutations are predominantly found as dominant mutations (Romano Ward Syndrome, RWS) and less frequently as homozygous recessive mutations (Jervell Lange-Neilsen Syndrome, JLNS) with hearing loss. Compound heterozygosity in single LQTS genes is reported in both RWS and JLNS. Reports of individuals with pathogenic mutations in different LQTS genes are difficult to find. Consequently, it is not known how mutations in multiple LQTS genes may affect phenotype. We describe a RWS family with heterozygous missense mutations in 3 different LQTS genes, including biallelic mutations at both the and subunits of IKs. The proband, an 8yo Caucasian male, diagnosed with RWS after 3 episodes of exercise-induced syncope. His mother had a cardiac arrest at age 17 and an AICD at age 29 for syncope. No other family members were symptomatic. The family was screened at 5 LQTS loci for mutations by exon-specific PCR, SSCP and DNA sequencing. Three mutations were identified in KCNQ1 and KCNE1, encoding IKs and subunits respectively, and in the subunit of IKr(KCNE2). These mutations were inherited in various combinations. Two of these (KCNE1-D76N & KCNE2-Q9E) have been associated with different forms of LQTS. The Q9E mutation has not previously been observed in Caucasians. The third mutation is novel, and resides in a highly mutable pore region of KCNQ1. This mutation was not found in 77 control samples, and exhibited concordance with the disease phenotype in this family. Molecular analysis revealed the following genotype-QTc correlations: proband, KCNQ1 +/- and KCNE1 +/-, (0.405 s); mother, KCNQ1 +/- and KCNE2 +/- (paced); father, KCNE1 +/- (0.433 s); sister 1, KCNQ1 +/- (0.472 s); sister 2, KCNE1+/-, KCNE2 +/- (0.467 s); sister 3, KCNQ1 +/-, KCNE1 +/- (0.478 s). Because the proband and sister 3 have normal hearing, we conclude that heterozygous mutation of both IKs subunits is not an obligatory JLNS genotype, and that sufficient normal IKs channels can be constituted in the presence of both mutations.

**Defective elastogenesis causing lethal pulmonary artery occlusion.** *M. Dasouki*<sup>1</sup>, *D. Markova*<sup>2</sup>, *R. Garola*<sup>3</sup>, *M-L. Chu*<sup>4</sup>. 1) Sec of Medical Gen & Molec Med, Children's Mercy Hospitals & CLinics, Kansas City, MO; 2) Department of Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, PA; 3) Department of Pathology, Children's Mercy Hospitals & Clinics, Kansas City, MO; 4) Department of Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, PA.

Disorders of elastogenesis may involve any of the proteins that comprise the microfibrillar and amorphous components of elastic fibers. Mutations involving fibrillin-1 and-2, elastin and fibulin-5 genes result in a broad spectrum of elastic tissue phenotypes affecting skin, skeleton, ocular and vascular structures. Frameshift mutations in the elastin gene have been reported in three families with autosomal dominant cutis laxa. A fibulin-5 gene homozygous missense mutation and a 22-kb internal duplication were reported in two autosomal recessive and autosomal dominant cutis laxa families respectively. A 27-day old Caucasian female had respiratory distress since birth requiring positive pressure ventilation. Family history was negative. were seen on echocardiography. Echocardiography showed dilated & tortuous pulmonary artery and aorta "causing tracheal compression", enlarged right ventricle and atrium and pulmonary hypertension. She also had mild cutis laxa and arachnodactyly. Skin biopsy was significant for markedly reduced elastic fibers. Her respiratory status continued to deteriorate due to the pulmonary vascular abnormalities and additional RSV infection. Post mortem examination showed biventricular hypertrophy, tortuous aneurysmal dilatation of ascending aorta and main pulmonary artery and wall dissection involving large segments of the great vessels with markedly reduced elastic fibers in the media. Skin fibroblast chromosomal and FISH analyses of the elastin gene were normal. DNA sequencing of the elastin and fibulin-5 genes showed an IVS3,+15 t->g heterozygous nucleotide change. RT-PCR products of the fibulin-5 mRNA transcript were of normal sizes excluding aberrant splicing. Northern analysis of mRNA for elastin, fibulin-2 and fibulin-5 showed a decreased fibulin-5 expression compared to control. This is of unknown significance.

**Localization of a familial cardiac conduction defect with adolescent onset to chromosome 3p21-p24. P.J.**

*Laitinen<sup>1</sup>, P. Mäkynen<sup>2</sup>, S. Yli-Mäyry<sup>2</sup>, V. Virtanen<sup>2</sup>, K. Kontula<sup>1</sup>, K. Aalto-Setälä<sup>2</sup>.* 1) Department of Medicine and Biomedicum Helsinki, University of Helsinki, Finland; 2) Tampere University Hospital, Finland.

Several inherited electrical cardiac diseases including the long QT syndrome type 3, Brugada syndrome, and both progressive and isolated form of cardiac conduction defect have been shown to result from mutations in the SCN5A gene which is located in chromosome 3p21-p24 and encodes a cardiac sodium channel. This study aimed to investigate whether in a large Finnish family an inherited cardiac conduction defect with onset in adolescence is linked to this chromosomal locus and whether SCN5A gene is involved in pathogenesis of the disease. A total of 44 family members were examined at the Department of Medicine, Tampere University Hospital. Affected individuals presented with progressive intracardial conduction delay, principally displayed by a widened QRS complex. Progressive atrio-ventricular (av) conduction delays up to a complete av-block appeared especially during exercise. The hearts of the affecteds were structurally normal. None of the affected individuals had any symptoms under the age of 12 years, but required pacemaker installation in their teens. No sudden cardiac deaths have been reported. Microsatellite markers D3S1298, D3S3527, D3S3623 and an intragenic SNP C5607T were genotyped in 15 family members (6 affected, 9 non-affected). In linkage analysis, lod score of 3.09 was obtained at marker D3S1298 and haplotype constructed of the markers co-segregated in the family with the disease but showed reduced penetrance. This indicates that SCN5A gene may play a role in this disease. Tan et al. (Nature 2001) reported a family with similar conduction defect and demonstrated a mutation in exon 12 of the SCN5A gene to underlie the phenotype. The main difference between the two families is the age at onset of symptoms: the index case described by Tan et al. was only 3 years of age while none of our patients have had their first symptoms before teenage. We are currently sequencing the SCN5A gene, but have not found mutations in the exons analyzed this far (including exon 12). In conclusion, SCN5A locus shows linkage to an exercise-induced conduction defect with onset in youth.

**Mutations in the SOX18 Transcription Factor Underlie Recessive and Dominant Forms of Lymphedema with Hypotrichosis and Telangiectasia.** *A. Irrthum*<sup>1</sup>, *K. Devriendt*<sup>2</sup>, *D. Chitayat*<sup>3</sup>, *G. Matthijs*<sup>2</sup>, *A. Mertens*<sup>2</sup>, *C. Glade*<sup>4</sup>, *P.M. Steijlen*<sup>4</sup>, *J.P. Fryns*<sup>2</sup>, *M.A.M. Van Steensel*<sup>4</sup>, *M. Vikkula*<sup>1</sup>. 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology & Université catholique de Louvain, Brussels, Belgium; 2) Center for Human Genetics, University of Leuven, Leuven, Belgium; 3) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Canada; 4) Department of Dermatology, University Medical Center Nijmegen, The Netherlands.

Hereditary lymphedema is a developmental disorder characterized by chronic swelling of the extremities due to a dysfunction of lymphatic vessels. Two responsible genes have been identified: VEGFR3, implicated in congenital lymphedema and FOXC2 causing lymphedema-distichiasis. Here we report three families with patients showing an unusual association of lymphedema, hypotrichosis and telangiectasia. Using microsatellite analysis, we first excluded both VEGFR3 and FOXC2 as causative genes. We then evaluated various murine phenotypes that present a combination of hair and cardiovascular anomalies including symptoms of lymphatic dysfunction as likely counterparts of the human disease. With this approach, we identified homozygous missense mutations in the gene encoding the SOX18 transcription factor in affected individuals from the two first families that present consanguinity. Two amino acid substitutions affect conserved residues in the first alpha-helix of the DNA-binding domain of the transcription factor. In the third family, both the affected child and his brother who died in utero presented a heterozygous nonsense mutation that truncates the protein in its trans-activation domain. This substitution was not found in the genomic DNA of either parent and hence constitutes a de novo germline mutation. This data shows that mutations in the SOX18 transcription factor gene cause both recessive and dominant lymphedema with alopecia and telangiectasia, suggesting that, in addition to its established role in hair and blood vessel development in mouse, the transcription factor is necessary for development and/or maintenance of lymphatic vessels.(vikkula@bchm.ucl.ac.be).

**Malignant familial cardiac fibrosis, without dilatation or hypertrophy, due to a novel exon-1 lamin A/C gene deletion: recognition of a distinct lamin related cardiomyopathy.** *W.S. Kerstjens-Frederikse<sup>1</sup>, J.P. van Tintelen<sup>1</sup>, R.A. Tio<sup>2</sup>, Y.J. Vos<sup>1</sup>, A.J.H. Suurmeijer<sup>3</sup>, L.G. Boven<sup>1</sup>, G.J. te Meerman<sup>1</sup>, M.P. van den Berg<sup>2</sup>, D.J. van Veldhuisen<sup>2</sup>, C.H.C.M. Buys<sup>1</sup>, R.M.W. Hofstra<sup>4</sup>, Y.M. Pinto<sup>5</sup>.* 1) Dept Clinical Genetics, Univ Hospital Groningen, Netherlands; 2) Dept Cardiology, Univ Hospital Groningen, Netherlands; 3) Dept Pathology, Univ Hospital Groningen, Netherlands; 4) Dept Medical Genetics, Univ Groningen, Netherlands; 5) Dept Experimental and Molecular Pathology, Univ Hospital Maastricht, Netherlands.

A large family with autosomal dominant cardiac fibrosis -without cardiac hypertrophy or dilatation- not fulfilling criteria of any known form of cardiomyopathy allowed us to identify a gene causing cardiac fibrosis. In affected family members, cardiac fibrosis manifested between 20 and 63 years of age by first degree AV block, (supra-) ventricular arrhythmias, rapidly progressive heart failure and a high rate of sudden death at relatively young ages. No neurological abnormalities were noted and echocardiography revealed neither dilatation nor (asymmetric) cardiac hypertrophy. Post mortem examination or myocardial biopsy in clinically affected subjects revealed massive interstitial fibrosis. Twelve family members were proven affected by histological evaluation. To identify the gene we collected DNA from consenting family members for linkage analysis studies. Genome wide linkage analysis yielded linkage in the vicinity of the LMNA gene, a LOD score of 3.46 at marker D1S1595 (5% recombination and 90% penetrance) with exclusion of other loci. The LMNA gene was analysed by DGGE and direct sequencing but no mutations were found. Subsequent Southern blot analysis of the gene revealed a deletion of exon 1 co-segregating with the disease. A non-penetrant 42-year-old family-member explained the lack of linkage with the LMNA locus itself. Conclusion: We describe a novel exon 1 deletion in the LMNA gene, that cannot be found by routine sequencing, which expands the phenotype of laminopathies with a non-dilating strictly fibrotic, yet highly malignant form of cardiomyopathy, not fulfilling the criteria for established forms of cardiomyopathy.

Program Nr: 2256 from 2003 ASHG Annual Meeting

**Mutation of Muscle LIM Protein is an Uncommon Genetic Cause for Familial Dilated Cardiomyopathy.** *D. Li, P. Jakobs, S.B. Parks, M. Bestwick, J. Kushner, E. Burkett, S. Ludwigsen, M. Litt, R. Hershberger.* Dept Medicine/Cardiology, Oregon Health & Sciences Unive, Portland, OR.

Familial dilated cardiomyopathy (FDC) is a primary cardiac muscle disease clinically characterized by enlarged cardiac chambers and decreased contractility, and commonly leads to heart failure and cardiac transplantation. FDC is usually inherited as an autosomal dominant trait. Although mutations in more than 10 genes have been identified using positional cloning or candidate gene approaches, the molecular cause of the majority of FDC remains unknown. These genes encode cytoskeletal or contractile proteins or their regulators. Muscle LIM protein (MLP) is enriched in striated muscle and is located in Z disc, a sarcomeric and cytoskeletal anchoring site. Mice with targeted disruption of the MLP gene exhibited a phenotype suggestive of dilated cardiomyopathy and heart failure. Thus, MLP was considered as a promising candidate gene for FDC. We sequenced the coding region of MLP in 92 FDC index cases; one mutation was identified in a 46 year old male with dilated cardiomyopathy (left ventricular ejection fraction 25%), atrial fibrillation and heart failure. The DNA sequence variant (T to C at nucleotide 55, GenBank NM\_003476) was predicted to substitute a highly conserved tryptophan at residue 4 with arginine (W4R). The missense mutation resulted in a charge change at the affected region of MLP and was predicted to alter the protein structure. This sequence variant was neither observed in other FDC index patients nor among 100 control chromosomes. Our finding provided further evidence to support a recent report that mutations in MLP though infrequent may cause FDC.

**Genetic study of Japanese patients with familial and sporadic primary pulmonary hypertension.** *T. Morisaki<sup>1,2</sup>, H. Morisaki<sup>1</sup>, S. Kyotani<sup>3</sup>, N. Nakanishi<sup>3</sup>*. 1) Dept Bioscience, Natl Cardiovasc Ctr Res Inst, Suita, Osaka, Japan; 2) Dept Molecular Pathophysiology, Osaka Univ Grad Sch Pharm Sci, Suita, Osaka, Japan; 3) Dept Cardiovasc Med, Natl Cardiovasc Ctr, Suita, Osaka, Japan.

Primary pulmonary hypertension (PPH) is a potentially lethal disorder, in which heterozygous mutations within the bone morphogenetic protein type II receptor (BMPRII) gene (BMPR2) have been identified. We report the molecular study of BMPR2 mutations in 3 Japanese families with familial PPH and in 31 Japanese patients with sporadic PPH as well as in 15 cases with secondary pulmonary hypertension (SPH). We have identified 11 mutations, including 2 missense mutations, distributed throughout the BMPR2 gene, in PPH but not in SPH. The majority of mutations are predicted to lead to a premature termination codon. Most of these mutations were novel and other additional synonymous nucleotide substitutions were also found. In all families with familial PPH studied, missense mutation or mutations resulting in premature termination of BMPR2 were found. In one case, the nucleotide substitution, resulting in a very short peptide of BMPRII terminated at the fourth amino acid residue, was found, strongly supporting that haploinsufficiency could account for the pathogenesis of PPH. We also tried to investigate genotype-phenotype relationships to elucidate the mechanisms contributing to pathogenesis of this important vascular disease. Regarding characteristics of nucleotide changes found in patients with PPH, there were similarities as well as differences between Japanese and other ethnic groups. These results indicate the considerable heterogeneity of BMPR2 mutations that cause PPH, and additional genetic and/or environmental factors will have to be identified.

**Mutation screening of the Thymopoietin gene (TMPO) in dilated cardiomyopathy.** *M. Taylor<sup>1</sup>, P. Fain<sup>1</sup>, E. Carniel<sup>1</sup>, A. Di Lenarda<sup>2</sup>, G. Sinagra<sup>2</sup>, M. Boucek<sup>3</sup>, L. Ku<sup>1</sup>, S. Graw<sup>4</sup>, J. Feiger<sup>1</sup>, D. Slavov<sup>1</sup>, X. Zhu<sup>1</sup>, D. Dao<sup>1</sup>, D. Feguson<sup>1</sup>, L. Mestroni<sup>1</sup>.* 1) Adult Medical Gen Program, Univ Colorado Health Sci Ctr, Denver, CO; 2) Division of Cardiology, University of Trieste, Italy; 3) Division of Cardiology, The Children's Hospital, Denver, CO; 4) Eleanor Roosevelt Institute, Denver, CO.

**Background:** Familial dilated cardiomyopathy (FDC) is a genetic disease caused by mutations in several FDC genes, including the lamin A/C gene (LMNA). Colocalization of and binding between the rat lamin-associated polypeptide-2 gene (LAP2) and LMNA gene products has been previously shown. The thymopoietin (TMPO) gene is the human counterpart to the rat lamin-associated polypeptide-2 gene (LAP2). Based on this data we believed TMPO to be a suitable candidate FDC gene and performed screening of TMPO in FDC families. **Methods:** Denaturing high-performance liquid chromatography heteroduplex analysis was used to screen 101 subjects from 75 families (49 FDC and 21 sporadic and 5 unclassified) with dilated cardiomyopathy (DCM). To date, over 90% of the coding region has been screened. **Results:** A putative TMPO mutation has been identified in one FDC pedigree in 2 severely affected male siblings. The older sibling developed cardiac symptoms at age 31 and was diagnosed at age 33 with a severe idiopathic DCM. The younger sibling presented with symptoms at age 22 and was diagnosed with idiopathic DCM at age 24. Neither subject had evidence of skeletal muscle dystrophy. In addition, a paternal cousin died unexpectedly at age 40. No additional family members were available for study. Both affected siblings inherited a CT transition in exon 4, encoding the TMPO alpha isoform, predicting an arginine (hydrophilic, charged residue) to cysteine (hydrophobic, uncharged residue) substitution at codon 690. The mutation is predicted to alter the C-terminal protein secondary structure (GOR IV software) and was absent in 300 control chromosomes (and a total of over 450 chromosomes). **Conclusion:** These results suggest that mutations in TMPO may represent a rare cause of FDC.

**Dilated Cardiomyopathy and the Microsatellite Polymorphism of Heme Oxygenase 1.** *J. Pohorence Ferguson, Y. Liu-Stratton, G. Cooke, P.F. Binkley.* The Ohio State University Davis Heart and Lung Institute, 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 (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 that reduces transcriptional activity. Sequence analysis was performed on 43 patients with ICM and 58 patients with DCM to identify the presence of this microsatellite polymorphism (L) that consists of 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 ( $p = 0.01$ ) higher proportion than ICM patients. An ELISA specific for the HO-1 protein was run on 14 DCM and 17 ICM sequenced patients, and the results showed that DCM patients with an L allele have lower HO-1 protein levels than DCM patients lacking an L allele ( $p = 0.11$ ). 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.

Etiology	L Absent	L Present
ICM	30	13
DCM	26	32

**Preliminary Results of Lamin A/C Mutation Screening in a Large Cohort of Familial Dilated Cardiomyopathy Patients.** *S.B. Parks, P. Jakobs, D. Li, J. Kushner, E. Burkett, S. Ludwigsen, M. Litt, R. Hershberger.* Department of Medicine/Cardiology, Oregon Health & Science University, Portland, OR.

Heart failure, which typically results from dilated cardiomyopathy (DCM), is responsible for 260,000 deaths per year in the US. One third or more of all patients with non-ischemic (idiopathic) dilated cardiomyopathy have a positive family history, referred to as familial dilated cardiomyopathy (FDC). Mutations in several genes have been linked to FDC, including lamin A/C (*LMNA*), -myosin heavy chain, cardiac troponin T, cardiac actin, desmin, and others. We have previously reported *LMNA* mutations in 3 of 16 FDC kindreds (19%). Other reports have suggested that *LMNA* mutations may account for 5-10% of FDC. It is still unclear what proportion of FDC cases are attributable to *LMNA*, or if a reliable genotype-phenotype correlation will emerge. In addition, mutations in *LMNA* can cause a variety of diseases, including Emery-Dreifuss muscular dystrophy, Charcot-Marie-Tooth disease, familial partial lipodystrophy and progeria, among others.

To better define the nature of *LMNA* mutations causing FDC, we have begun a mutation screening protocol in 122 of our FDC kindreds. With approximately 7% of the protocol completed, we have identified a novel *LMNA* missense mutation, a G to C transversion at nucleotide 302. The G302C mutation is predicted to replace the wildtype arginine with proline at residue 101 within the central rod domain of the protein. This domain, an helical coiled coil, is formed by hydrophobic residues in the center and charged amino acids on the surface. Replacement of a positively charged arginine with a hydrophobic proline is expected to have a dramatic affect on polypeptide structure, and to likely alter protein function. The subject carrying the G302C mutation developed DCM and atrial fibrillation in his 30s, progressing to heart failure and eventual cardiac transplantation. His brother and at least four other relatives have been similarly affected. We anticipate that the discovery of this and other FDC associated *LMNA* mutations in our cohort will help to advance the status of mutation detection in dilated cardiomyopathy.

***COL3A1* mutations in Japanese patients with Ehlers-Danlos syndrome type IV (EDSIV).** A. Watanabe<sup>1,2</sup>, N. Sakai<sup>1</sup>, T. Wada<sup>3</sup>, M. Fujimoto<sup>4</sup>, Y. Fukushima<sup>3</sup>, T. Shimada<sup>1,2</sup>. 1) Dept Molec & Medical Genet, Nippon Medical Sch, Tokyo, Japan; 2) Div Clinical Genet, Nippon Medical Sch Main Hosp, Tokyo, Japan; 3) Div Clinical & Molecular Genet, Shinshu Univ Hosp, Nagano, Japan; 4) Dept Dermatology, Jichi Medical Univ, Tochigi, Japan.

Ehlers-Danlos syndrome type IV (EDSIV), the vascular type, is an autosomal dominant disorder and results from mutations in the genes for type III procollagen (*COL3A1*). Affected patients are at risk for arterial, bowel, and uterine rupture.

We analyzed four unrelated Japanese patients with the features of EDSIV. In order to identify mutations in patients RNA, the 3.3 kb reverse transcriptase polymerase chain reaction (RT-PCR) product containing triple-helical domain of *COL3A1* was prepared from cultured skin fibroblast and sequenced directly. Our results showed four heterozygous mutations, three novel missense base substitutions (Gly220Trp, Gly727Asp, Gly877Asp) and a 24 bp insertion mutation in 1440th nt of position of *COL3A1*. In this insertion mutation, eight amino acids added to in frame *COL3A1* but had no glycine, supposed to be decreased the normal mature type III collagen. Further analysis of the genomic structure of the insertion type is in progress. We also discuss the genotype-phenotype correlations in these individuals.

**Role of PTEN in lipid signaling in breast cancer.** *C. Alvarez-Breckenridge, K.A. Waite, C. Eng.* Human Cancer Genetics, The Ohio State University, Columbus, OH.

The tumor suppressor gene PTEN encodes a dual specificity phosphatase that recognizes both protein and phosphatidylinositol lipid substrates and modulates cellular functions such as migration and proliferation. Germline, but more importantly somatic, mutations in PTEN have been shown to play a role in breast cancer. Since its discovery, the role of PTEN as a negative regulator of the phosphatidylinositol-3-kinase/Akt pathway has been intensely investigated. In contrast, little is known about the role of PTEN in regulating other lipid signaling pathways by modulating phosphatidylinositol(4,5)bis-phosphate (PIP<sub>2</sub>; PTENs product) and phosphatidylinositol(3,4,5)tri-phosphate (PIP<sub>3</sub>; PTENs substrate). Within the last two decades, and underscored by the discovery that PTEN is a lipid phosphatase, phospholipids have been shown to play a critical role in signal transduction, and are key players in determining whether a cell divides. With the continued work of lipid signaling events and metabolism, it is becoming clear that the modulation of PIP<sub>2</sub> and PIP<sub>3</sub> levels is a theme in cellular regulation. As a consequence, PTEN may be a master regulator of cellular regulation due to its ability to modulate the levels of these lipids. Based upon these findings, we hypothesize that PTEN regulates lipid signaling and lipid homeostasis. We found that in unstimulated MCF-7 cells, increased expression of PTEN, through an inducible vector, results in a 51% increase in phosphatidic acid (PA), a product of phospholipase D (PLD), suggesting that PTEN may regulate PLD. Further, overexpression of PTEN results in a 1.3-fold increase in PLD activity in unstimulated cells, with a concomitant decrease in phosphatidylcholine, the PLD substrate. This activation appears to be dependent upon the lipid phosphatase activity as overexpression of mutated PTEN protein that does not have lipid phosphatase activity results in a decrease in basal PLD activity. Further work along these lines will allow us to understand the role of PTEN in lipid-mediated signaling events and may provide novel targets for cancer therapy and prevention.

**Functional exploration of A type lamins and associated proteins in patients affected with Hutchinson-Gilford Progeria Syndrome caused by G608G mutation in LMNA.** *N. LEVY*<sup>1,2</sup>, *C. Navarro*<sup>1</sup>, *I. Boccaccio*<sup>1</sup>, *A. Boyer*<sup>2</sup>, *P. Negre*<sup>2</sup>, *K. Devriendt*<sup>3</sup>, *Y. Shafeghaty*<sup>4</sup>, *J. Bridger*<sup>5</sup>, *N. Philip*<sup>2</sup>, *R. Bernard*<sup>2</sup>, *F. Leturcq*<sup>6</sup>, *M. Lemerrer*<sup>7</sup>, *P. Cau*<sup>1,8</sup>, *A. DeSandre-Giovannoli*<sup>1</sup>. 1) Inserm U491, Faculte de Medecine, Marseille, France; 2) Departement de Genetique Medicale, Hopital d'enfants de la Timone, Marseille, France; 3) Centrum voor menselijke erfelijkheid, Leuven, Belgium; 4) Genetics Research Center, Tehran, Iran; 5) Cell and Chromosome Biology Group, Dept. of Biological Sciences, Brunel University, UK; 6) Laboratoire de biochimie gntique, hopital Cochin, Paris; 7) Inserm U393, Hopital Necker, Paris, France; 8) Laboratoire de Biologie Cellulaire, Hopital Conception, Marseille, France.

Progeria is a rare, typical and severe premature aging disorder. Death usually occurs around 13.4 years, due to stroke or coronary artery disease. Recently, we and others identified the a single point mutation in LMNA encoding Lamin A as the cause of progeria. To further explore the mechanisms underlying progeria, we performed genomic transcriptional and functional analysis in 7 patients affected with HGPS. The c.1824C>T (p.G608G) mutation was detected in all cases. Transcript splicing and/or protein expression levels for lamins A/C, lamins B and emerin were analyzed by RT-PCR and western blotting on patients lymphoblasts or fibroblasts. In all cases, a 150 bp heterozygous deletion was identified due to the aberrant use of a cryptic donor splice site. Western blotting showed a decreased amount of Lamin A with or without truncated Lamin A. Immunocytochemistry with antibodies to Lamin A/C, A, B1, B2, emerin and nup62 was performed. Nuclear sizes were highly variable; herniations and interruptions of the nuclear envelopes with chromatin extrusion were observed. Lamin A/C and emerin were often lacking on one pole of the nuclei, being delocalized to the nucleoplasm into foci or with homogeneous patterns. Lamins B were delocalized as voluminous foci in the nucleoplasm. These findings underscore the implication of perturbed nuclear functions in the pathogenesis of progeria and orientate the research towards specific molecular interplays being impaired.

**Defining Branchio-Oto-Renal syndrome and determining the contribution of EYA1 mutations to this phenotype.**

*E.H. Chang*<sup>1</sup>, *N.C. Meyer*<sup>1</sup>, *V.S. Vervoort*<sup>2,3</sup>, *C.E. Schwartz*<sup>2,3</sup>, *R.J.H. Smith*<sup>1</sup>. 1) Molecular Otolaryngology, University of Iowa, Iowa City, IA; 2) Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29654; 3) Greenwood Genetic Center, Greenwood, SC 29646.

Background: BOR syndrome is an autosomal dominant disorder with an estimated prevalence of 1:40,000. It affects ~2% of profoundly deaf children and has a variable phenotype that typically involves the branchial arches, ears, and kidneys. EYA1 mutations are known to cause BOR syndrome and are reported in ~18% of families segregating a BOR phenotype. Objective: To refine the diagnostic criteria for BOR by analyzing phenotypic data from 33 families in which disease-causing EYA1 mutations have been found; to develop a novel screening method to detect large insertions/deletions of EYA1. Methods: Literature review of phenotypic data from persons with documented EYA1 mutations and the presentation of 9 novel disease-causing mutations and their phenotypes. Intervention: Mutation screening of EYA1 was completed on DNA by single-strand conformational polymorphism (SSCP) analysis. Samples were sequenced bi-directionally if shifts were observed on MDE gels. A semi-quantitative based screen was developed to detect large insertions and deletions in EYA1, which were confirmed via sequence-based or single nucleotide polymorphism (SNP) analysis. Results: Of 33 disease-causing EYA1 mutations with adequate phenotypic data, 16 are nonsense or frameshift, 8 are missense, 6 are splice site, and 3 are large deletions. The most common resulting phenotype includes deafness (98.5%), preauricular pits (83.6%), branchial anomalies (68.5%), renal anomalies (38.2%) and external ear abnormalities (31.5%). Based on these phenotypic data, we developed strict diagnostic criteria for BOR syndrome and found that 30% of families fulfilling these criteria had mutations in EYA1 detectable by SSCP analysis. Our semi-quantitative PCR screen identified an additional 4 of 17 patients with large insertions/deletions of EYA1. This result means that ~50% of persons with a BOR phenotype will have mutations in EYA1, although 40% of the identified EYA1 mutations will be large insertions/deletions.

**Further evidence that a tremor/ataxia syndrome may occur in Fragile X premutation carriers: findings from two**

**clinical series.** *E. Di Maria*<sup>1</sup>, *M. Grasso*<sup>2</sup>, *S. Pigullo*<sup>1</sup>, *F. Faravelli*<sup>2</sup>, *G. Abbruzzese*<sup>1</sup>, *P. Barone*<sup>3</sup>, *P. Martinelli*<sup>4</sup>, *S. Ratto*<sup>5</sup>, *R. Sciolla*<sup>6</sup>, *E. Bellone*<sup>1</sup>, *F. Dagna-Bricarelli*<sup>2</sup>, *F. Ajmar*<sup>1</sup>, *P. Mandich*<sup>1</sup>. 1) Dept Neurosci Ophthalmol Genet, Univ Genova, Italy; 2) SC Lab Hum Genet, EO Ospedali Galliera, Genova, Italy; 3) Dept Neurol Sci, Univ Napoli Federico II, Italy; 4) Inst Clinical Neurol, Univ Bologna, Italy; 5) SC Neurol, EO Ospedali Galliera, Genova, Italy; 6) Div Neurol, Ivrea Hospital, Ivrea, Italy.

Fragile X (FraX) premutation, defined as the presence of 55-200 CCG repeats in the FMR1 gene, is known to contribute to the FraX phenotype through expansion to full mutation allele (>200 CCG). There is emerging evidence that premutation alleles may cause in older male carriers a syndrome presenting with intention tremor, ataxia and a peculiar neuroimaging pattern. We examined for FraX premutation two series of clinically ascertained male patients: i) 114 subjects (belonging to 71 families) diagnosed as having definite essential tremor (ET) (mean age 62.5, SD 17.7); ii) 51 individuals (48 families; mean age 57.8, SD 15.1) referred as having spinocerebellar ataxia (SCA), who resulted negative for SCA1, SCA2, SCA3, SCA6, DRPLA repeat expansions. No premutation allele was found in the ET cohort (82 patients were older than 50 years). Conversely, we identified two SCA patients carrying the premutation (84 and 86 repeats, respectively). This corresponds to a rough 4% proportion (2/51), which raises up to 7.1% if only subjects older than 50 are included (2/28). The first patient (aged 55 at examination) presented with gait ataxia, dysarthria, muscle weakness and atrophy of lower limbs. The second patient (71 ys.) had severe kinetic tremor, along with cerebellar atrophy and signal alteration of the middle cerebellar peduncles on MRI. These observations were consistent with the clinical profile reported in affected male carriers. In summary, these findings allowed to rule out FraX premutation as genetic risk factor for ET. On the other hand, the excess of premutation carriers among SCA individuals further confirms that the presence of a premutation allele contribute to a distinct movement disorder and should be investigated in patients with such a clinical presentation.

**Three novel SACS mutations in Italian patients with Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay type.** *C. Casali<sup>1</sup>, G. Grieco<sup>2</sup>, A. Malandrini<sup>3</sup>, M. Valoppi<sup>1, 2</sup>, G. Comanducci<sup>1</sup>, V. Leuzzi<sup>4</sup>, S. Palmieri<sup>3</sup>, L. Benedetti<sup>1</sup>, A. Pierallini<sup>5</sup>, A. Tessa<sup>6</sup>, S. Gambelli<sup>3</sup>, R. Pierini<sup>6</sup>, A. Federico<sup>3</sup>, F. Pierelli<sup>1, 7</sup>, E. Bertini<sup>6</sup>, F. Santorelli<sup>1, 2</sup>.*  
1) Dept Neurology & ORL, La Sapienza Univ, Rome, Italy; 2) Molecular Neurogenetics, IRCCSC Mondino, Center of Experimental Neurobiology Mondino-Tor Vergata-S.Lucia, Rome, Italy; 3) Institute of Neurological Sciences, University of Siena, Siena, Italy; 4) Dept Child Neurology and Psychiatry, La Sapienza University, Rome, Italy; 5) Dept Neurological Sciences, La Sapienza University, Rome, Italy; 6) Molecular Medicine, IRCCS-Bambino Gesù, Rome, Italy; 7) IRCCS-Neuromed, Pozzilli (IS), Italy.

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is an early-onset form of cerebellar ataxia with spasticity and axonal neuropathy, which occurs frequently in the Charlevoix-Saguenay-Lac-Saint-Jean region of Quebec, but is believed to be rare elsewhere. ARSACS is associated with mutations in the SACS gene, which encodes saccin, a protein of unknown function. In two Italian ARSACS families, we identified three novel SACS mutations resulting in premature translation termination of saccin. Apart from the absence of retinal striation, the phenotype of the Italian patients closely resembles that of French Canadian cases. While lending definitive support to the notion that SACS mutations are not confined to the Quebec region, our data also expand the number of genetic alterations that must be considered in patients with cerebellar ataxia.

**Fibroblast expression profile analysis in the human progeroid syndrome (MAD) associated to LMNA mutations.**

*F. Amati<sup>1</sup>, M. Biancolella<sup>1</sup>, M.R. D'Apice<sup>1</sup>, S. Gambardella<sup>1</sup>, R. Mango<sup>1</sup>, P. Sbraccia<sup>2</sup>, M. Federici<sup>2</sup>, M. D'Adamo<sup>2</sup>, A. Nardone<sup>1</sup>, K. Margiotti<sup>1</sup>, M. Lewis<sup>3</sup>, G. Novelli<sup>1</sup>.* 1) Dept Hum Genetics, Tor Vergata Univ, Rome, Italy; 2) Dept Int Medicine, Tor Vergata Univ; 3) Dept Psychology, Univ of Texas at Austin, USA.

MAD (Mandibuloacral dysplasia OMIM\*248370) is a rare autosomal recessive disorder characterized by mandibular and clavicular hypoplasia, acroosteolysis, delayed closure of cranial sutures, joint contractures, mottled cutaneous pigmentation and partial lipodystrophy. MAD is caused by a mutation in the LMNA gene (R527H). The LMNA gene is also implicated in a wide spectrum of genetic disorders called laminopathies. It is reasonable that laminopathies arise from alteration and/or disturbance in differentiation, maintenance, repair or regulation of mesenchymal cells. Three possible mechanisms have been suggested to explain the extreme phenotypic variability induced by LMNA mutations, such as nuclear fragility, alteration of gene expression patterns, and knock-on effect on the complement of proteins found in the endoplasmic reticulum. To understand the molecular pathogenesis of this disorder, we performed an expression analysis of 5184 genes using a cDNA microarray (DermArray, Integriderm). Cultured fibroblasts were obtained from skin specimens derived from two patients (a 37 yrs old male and a 22 yrs old female) and from three control subjects (a 40 yrs man and two females with a mean age of 21 yrs old). Microarray analysis was performed by Pathways 4-Universal Microarray Software (ResGen). A total of 119 genes show a differential expression in MAD fibroblasts. Fifty genes were upregulated (ratio > 3) and 69 downregulated (ratio < 0,3). Only 15 of them showed a common behavior in both patients. The six upregulated genes include structural, ribosomal and carrier proteins; the nine downregulated genes encode for enzymes, transcription factor and structural, contractile and heat shock proteins. The altered expression of 8 of these 15 genes was confirmed by Q-RT PCR. Among the upregulated genes, we found COL3A1, while among the downregulated genes we detected POLR2B. Both these proteins could play an important role in MAD pathogenesis.

**Expression of the Hutchinson Gilford Progeria Mutant Lamin A Causes Abnormal Nuclear Morphology and Lamin A Mislocalization.** *M.W. Glynn, T.W. Glover.* Dept of Human Genetics, Univ of Michigan, Ann Arbor, MI.

Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare disease that affects children in the first decade of life and causes a remarkable phenotype resembling many aspects of aging. Affected children have an aged appearance, a lack of subcutaneous fat, growth retardation and severe atherosclerosis. The average life span of progeria patients is 13 years, with most dying from complications due to atherosclerosis. Virtually all HGPS patients have the same mutation creating an abnormal splice donor site in exon 11 of the LMNA gene. The result of the mis-splicing creates a protein missing 50 amino acids near the C-terminus. The deleted region includes a protein cleavage site that normally removes 18 amino acids including a CAAX box farnesylation site. The LMNA gene codes for lamin A and lamin C proteins, which are a component of the nuclear lamina, a fibrous matrix located at the interior of the nuclear membrane, responsible for nuclear integrity and organization. We have created GFP-tagged normal and HGPS-mutant lamin A expression constructs and have transfected normal and HGPS fibroblasts. As expected, the normal protein localizes at the periphery of the nucleus in normal cells. Expression of the GFP-tagged mutant lamin A in normal fibroblasts causes an abnormal nuclear morphology including mislocalization of the GFP-tagged mutant lamin A to foci at the nuclear periphery, and nuclear blebbing similar to that seen in HGPS fibroblasts. Expression of normal lamin A in HGPS fibroblasts did not significantly correct the nuclear blebbing phenotype. Furthermore, the normal GFP-lamin A was mislocalized indicating that the presence of the endogenous mutant can cause mislocalization of the normal GFP-tagged lamin A in a dominant-negative fashion. These results show that the mutant HGPS cellular phenotype can be recreated in normal cells and that the mutant phenotype is not easily corrected by over-expression of the normal protein. In addition, they provide evidence for the mislocalization of the HGPS-specific form of lamin A, which may affect lamin A function in an array of nuclear processes including transcription, replication and cell cycle functions.

**Phenotypic variability in Friedreich ataxia: spastic paraplegia in the absence of point mutations in the FRDA gene.** *E. Andermann*<sup>1,2</sup>, *A. Badhwar*<sup>1,2</sup>, *M.H. Seni*<sup>1,2</sup>, *M. Pandolfo*<sup>3</sup>, *F. Andermann*<sup>2</sup>. 1) Neurogenetics Unit, McGill Univ, Montreal, PQ, Canada; 2) Department of Neurology and Neurosurgery, Montreal Neurological Hospital and Institute, McGill Univ, Montreal, PQ, Canada; 3) Centre Hospitalier de l'Universit de Montral, Montreal, PQ, Canada.

Friedreich ataxia (FA) is an autosomal recessive disease with onset before the age of 25. The majority of FA cases are due to mutations in the Frataxin gene (FRDA) on 9q13. 96% of patients with FA have homozygous expansions of an intron 1 GAA repeat. 4% have an expansion on one allele and a point mutation on the other. Point mutations (particularly D122Y and G130V) may give rise to an atypical FA phenotype with slow disease progression, minimal or no ataxia, and gait spasticity (Durr et al, 1999). We report a male patient with late onset, slowly progressive spastic paraplegia, in a FA family with 3 affected siblings and marked intra-familial phenotypic variability. The 43-year old proband(P1) first presented with cardiomyopathy at age 16. He developed progressive spasticity, bilateral lower limb weakness and mild ataxia at age 25, and was wheelchair-bound at age 40. His sister(P2) was diagnosed with FA at age 29, exhibiting ataxia, bilateral lower limb weakness and dysarthria. She initially presented with insulin dependent diabetes at age 21. His brother(P3) developed progressive ataxia and leg weakness in early childhood. By age 24, he had developed diabetes, optic atrophy, hearing loss and cardiomyopathy. He died at age 31. Autopsy revealed slight atrophy of the cerebellum at the level of the vermis and atrophy of the dorsal roots. Mutation analysis in P1, 2 and 3 confirmed the diagnosis of FA in this family. P1 had expansions in both FRDA alleles, each with 750 GAA repeats. P2 had 850 and 450 repeats, while P3 had 1050 and 790 repeats. Marked intra-familial phenotypic variability with respect to age of onset and mode of presentation exists in this FA family, despite average size GAA expansions in both alleles, demonstrating the importance of modifying genes. Our proband is unusual in having atypical FA with spastic paraplegia with equal GAA expansions in both alleles and without point mutation in D122Y and G130V.

**Functional studies of the RNA component of RMRP in Cartilage Hair Hypoplasia.** *P. Hermanns*<sup>1,3</sup>, *A. Bertuch*<sup>1</sup>, *M. Schmitt*<sup>4</sup>, *B. Zabel*<sup>3</sup>, *B. Lee*<sup>1,2</sup>. 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute; 3) Children's Hospital, University of Mainz, Germany; 4) Biochemistry and Molecular Biology, SUNY Upstate Medical University, Syracuse, NY.

RNase mitochondrial RNA processing endoribonuclease (RMRP) is a nuclear encoded, ubiquitously expressed pol III RNA transcript. It is part of a large ribonuclear protein complex with multiple functions in yeast including processing of the pre-rRNA, cleavage of mitochondrial RNA for priming DNA replication, and perhaps cell cycle control. However, its function in vertebrate development is completely unknown. Mutations in RMRP cause a skeletal malformation syndrome CHH affecting chondrogenesis, hematopoiesis, and hair development. Four single base pair substitutions were found in our cohort of CHH patients (70AG, 124CT, 180GA, 262GT) and while the fifth mutation was a 2 bp deletion mutant (267,268). We have introduced these five mutations in the essential RMRP yeast ortholog NME1 to understand effects of human mutations on NME1 function. The *nme1* mutants were all viable and their growth rates were indistinguishable from wild type control, with the exception of the 2 bp deletion, which showed a significant growth delay. Since RMRP is involved in mitochondrial replication we also looked for loss of mitochondrial function in the mutant strains at different temperatures. No differences were found in mutant vs. wild type strains. Furthermore, we did not detect evidence of cell cycle delay in four of the *nme1* mutants via FACS analysis. RNase MRP is required for production of the 5.8S<sub>S</sub> rRNA species. Mutations in yeast cause failure to process the 5.8S<sub>L</sub> rRNA to 5.8S<sub>S</sub> rRNA. Two of the *nme1* mutants (70AG and the 2bp deletion mutant) showed an alteration of the 5.8S<sub>S</sub>/5.8S<sub>L</sub> rRNA ratio. These data show that a subset of CHH mutations can affect ribosomal RNA processing while more severe mutations are likely lethal. Together these studies will provide insight into the pathogenesis of the pleiotropic effects observed in CHH.

**Causative Malignant Hyperthermia mutations excluded in the pathophysiology of South African clubfoot**

**patients.** *C. Mouton*<sup>1</sup>, *B. Semete*<sup>1</sup>, *H. Brand*<sup>2</sup>, *A. Olckers*<sup>1,3</sup>. 1) Centre for Genome Research, Potchefstroom University for CHE, Pretoria, South Africa; 2) Department of Anesthesiology, University of Pretoria, South Africa; 3) DNAbiotec (Pty) Ltd, Pretoria, South Africa.

Malignant hyperthermia (MH) is a pharmaco-induced syndrome, characterised by skeletal muscle calcium dysregulation, hypermetabolism and elevated body temperatures. This autosomal dominant genetic predisposition is a response to individual exposure to inhalational anaesthetics or depolarising muscle relaxants. If not treated immediately, MH results in severe tissue damage, organ failure and death. Although treatment has led to a significant decline in mortality associated with MH, it still remains the most common cause of death from general anaesthetic. At present the diagnosis of MH based on clinical phenotypes alone is invasive, and cumbersome. For this reason the molecular diagnosis of this disorder needs to be embraced. Various mutations in the skeletal muscle ryanodine receptor (RYR1) gene located on chromosome 19q13.1, and encoding the calcium release channel of skeletal muscle, can account for susceptibility to MH. A number of mutations have been reported within the RYR1 gene, although only seventeen have been demonstrated to be causative. This study included three infants, aged three to eight months, who had typical clubfoot phenotypes and a Clinical score of six (performed according to Larach et al., 1994). With their first exposure to anaesthesia which was during surgery for clubfoot, these patients developed an MH episode. The anaesthetic agents included Sevoflurane or Halothane. All three patients were screened for seventeen causative mutations via RFLP and automated sequencing. None of the mutations investigated were detected in these individuals. This result was not unexpected, although, these mutations had to be excluded. Their absence implies that these seventeen mutations do not contribute towards the MH phenotype of these three clubfoot patients. This study is the first to investigate the link between the clubfoot and MH phenotypes concurrently observed in this group of patients.

**Examination of the mitral valve prolapse locus on chromosome 11p15.4.** *M. Leyne*<sup>1</sup>, *F. Nesta*<sup>1,2</sup>, *D. Dai*<sup>1</sup>, *J. Acierno*<sup>1</sup>, *L. Freed*<sup>1,2</sup>, *R. Levine*<sup>1,2</sup>, *S. Slaugenhaupt*<sup>1,2</sup>. 1) Harvard Inst Human Genetics, Massachusetts General Hosp, Boston, MA; 2) Harvard Medical School, Boston, MA;

Mitral valve prolapse (MVP) is a very common cardiac disorder in humans, occurring in approximately 2.4% of the general population. The condition is characterized by systolic billowing of the mitral leaflets into the left atrium, often accompanied by mitral regurgitation. Early attempts at establishing a genetic basis for MVP were limited by difficulties in diagnosis; however, recent advances in echocardiography have significantly increased the diagnostic specificity. We have recently identified a novel MVP locus, *MMVP2*, on chromosome 11p15.4 by genetic linkage mapping in a single large pedigree. The *MMVP2* locus was originally defined as a 4.3-cM region between the markers *D11S1923* and *D11S1331*. This region contained several large sequence gaps and 46 RefSeq genes. Initial examination of the known genes in the large candidate interval revealed a potential candidate, matrix metalloproteinase-26 (*MMP26*). *MMP26* was screened for mutations since MMPs are known to function in the degradation of the extracellular matrix and several MMPs have been shown to be over-expressed in myxomatous valve tissue. No coding sequence or splice site changes were identified in MVP patients in this family, suggesting that this gene is not likely to be involved in MVP. We have now used haplotype analysis with new informative genetic markers to narrow the candidate interval from 4.4 Mb to 2.7 Mb between the markers *PNM1104* and *D11S1331*. This smaller region has no sequence gaps and contains only 38 RefSeq genes, which are currently being prioritized and screened for MVP mutations. Determination of the genetic basis of MVP is important because the disease often manifests clinically in the 5th or 6th decade of life through presentation as a severe cardiac event. Earlier intervention in genetically susceptible individuals could potentially prevent progression to a clinically severe stage.

**Human spastin (SPG4): Functional characterization of potential nuclear localization sequences and N-terminal deletion mutants.** *J. Schickel<sup>1</sup>, M. Brodhun<sup>2</sup>, K. Moutzouris<sup>1</sup>, T. Deufel<sup>1</sup>.* 1) Inst Klinische Chemie, FSU Jena, Jena, Germany; 2) Inst Pathologie, FSU Jena, Jena, Germany.

The SPG4 locus at 2p21-p22 has been shown to account for approximately 40-50 percent of all families with autosomal dominant hereditary spastic paraplegia (HSP); the SPG4 gene is ubiquitously expressed in adult and fetal tissues and encodes for spastin, an 616-amino-acid ATPase belonging to the AAA protein family. It was shown that overexpressed wild-type spastin interacts transiently with microtubules and is likely to be involved in microtubule dynamics; in this model, spastin association with the microtubule cytoskeleton is mediated by the N-terminal region of the protein and regulated through the ATPase activity of the AAA domain. In a recent report, however, immunolabeling experiments using polyclonal antibodies suggest that spastin protein is localized in the nucleus outside the nucleolus. Our study was aimed at characterizing functional domains in the N-terminal part of spastin, specifically 1) testing the functionality of putative nuclear localization sequences (NLS), and 2) mapping the tubulin-binding region. Constructs containing four predicted NLS fused to green fluorescent protein (GFP) were generated and transiently expressed in cultured cells. Our results indicate that none of these sequences in neither C-terminal nor N-terminal fusions was sufficient for nuclear targeting. A set of deletion mutants of both wild-type spastin and the K388R mutant spastin, the latter known to irreversibly associated with microtubule, were fused to GFP and transfected. Binding to MT is tested by immunofluorescence and will allow to fine-map the MT interacting domain.

**Identification of two novel mutations of the human elastin gene in Korean patients with SVAS.** *S.M. Park<sup>1</sup>, E.J. Seo<sup>1,2</sup>, Y.H. Kim<sup>1</sup>, H.W. Yoo<sup>1,3</sup>, Y.H. Kim<sup>1,3</sup>, J.K. Ko<sup>1,3</sup>, I.S. Park<sup>1,3</sup>.* 1) Genome Research Center for Birth defects and Genetic disorders, Asan Medical Center, Seoul, Korea; 2) Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea; 3) Department of Pediatrics, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.

The human elastin gene (ELN) has 34 exons and spans an approximately 45 kb of genomic region at chromosome 7q11.23, encoding tropoelastin, an elastin precursor of 72 kDa. Elastin is composed largely of glycine, proline, and other hydrophobic residues and contains multiple lysine-derived crosslinks, such as desmosines, which link the individual polypeptide chains into a rubber-like network. The resilience of many tissues, such as vascular walls, skin, ligaments, and lungs are maintained by elastic fibers in the extracellular matrix. Genetic defects of ELN are known to be associated with the development of supravalvular aortic stenosis (SVAS), a cardiovascular disease with the characteristic thickening of the aorta media and intima. We have identified two novel mutations of ELN, 890\_922del and 2260C>A, in two unrelated Korean patients with SVAS. The 890\_922del mutation causes deletion of 11 amino acids at exon 17, which corresponds to the hydrophilic region involved in cross-linking of this protein. The 2260C>A mutation substitutes Trp for Arg at codon 754 in exon 34, which encodes a hydrophobic domain. Functional characterization of these mutations will be helpful in understanding the molecular causes of SVAS and, furthermore, in developing therapeutic treatments of this cardiovascular disease.

**Muscle proteins alteration in patients with mutations in the Fukutin-Related Protein gene.** *L.U. Yamamoto, M. Canovas, R.C.M. Pavanello, B.L. Lima, F. de Paula, N. Vieira, M. Zatz, M. Vainzof.* Human Genome Research Center, IBUSP, University of Sao Paulo, SP, Brazil.

The fukutin-related protein (FKRP) has been recently identified, with sequence similarities to a family of proteins involved in the glycosylation of cell surface molecules. Mutations in the FKRP gene were identified in limb-girdle muscular dystrophy type 2I (LGMD2I) and congenital muscular dystrophy type 1C (CMD1C), both previously mapped to an identical region on chromosome 19q13.3. CMD is characterized by onset of symptoms within the first few months of life, and in the 1C form, there is inability to walk. LGMD2I has a milder and variable course, a slower progression, and the age at onset varying from the first to the fourth-fifth decade of life. Both show preserved intelligence and elevated serum CK. Secondary protein abnormalities such as a variable reduction in the expression of  $\alpha$ -dystroglycan and 2-laminin have been described in the CMD1C form. Here we report the results of the analysis of muscle proteins associated to the sarcolemma (dystrophin, sarcoglycans, dysferlin) extracellular matrix (2-laminin, collagen VI), from the sarcomere (telethonin) and from the muscle cytosol (calpain 3) in muscle biopsies from 13 unrelated patients with molecular diagnosis of LGMD2I. We observed that eight patients, five with the common C826A mutation, showed a variable degree of deficiency of 2-laminin, through immunofluorescence analysis; three patients showed calpain 3 and two patients, deficiency of dystrophin, through western blot analysis. The present study shows that in our population, mutations in the FKRP gene can lead to secondary reduction of several muscle proteins, and the deficiency of 2-laminin on sections is clearly prevalent in the milder form. Financial support: FAPESP-CEPID, PRONEX, CNPq.

**Deficiency of myotilin in skeletal muscle of a patient with limb-girdle muscular dystrophy: a primary or a secondary defect?** *M. Vainzof<sup>1</sup>, L.U. Yamamoto<sup>1</sup>, P.M. Kossugue<sup>1</sup>, L.L.Q. Fogaca<sup>1</sup>, J. Gurgel-Giannetti<sup>2</sup>, M. Moza<sup>3</sup>, P. Salmikangas<sup>3</sup>, O. Carpen<sup>3</sup>.* 1) HGRC-IBUSP, University of Sao Paulo, SP, Brazil; 2) UFMG, Belo Horizonte, Brazil; 3) Dep of pathology, University of Helsinki, Helsinki, Finland.

Myotilin is a thin filament-associated Z-disc protein, that binds to -actinin, actin and filamin C. Mutations in the myotilin gene lead to a rare autosomal dominant limb-girdle muscular dystrophy 1A form (LGMD1A), characterized by proximal muscle weakness, a dysarthric speech and high serum CK levels. On muscle biopsy myopathic alterations and rimmed vacuoles are observed. Up to now, only two families with T57I and S55F missense mutations have been described; the former mutant allele is transcribed and normal levels of correctly localized myotilin protein are seen. Here we identified a 14-year-old patient, with severe proximal muscle weakness, with difficulties to climb stairs, to get up from the floor, or to raise the arms. Serum CK is 15X increased and EMG is myopathic. Muscle biopsy analysis showed degeneration, variation in fiber size, fiber splitting, connective tissue replacement and a large number of rimmed vacuoles. Muscle protein analysis revealed normal expression for dystrophin, sarcoglycans, calpain 3, dysferlin, telethonin and 2-laminin. On the other hand, western blot analysis using three different myotilin antibodies showed a clear reduction in the amount of the 57 kDa myotilin band, in comparison to other cytoskeletal proteins and to control muscle biopsies. Lymphocyte DNA analysis for the myotilin gene is ongoing to verify if this deficiency is a primary or a secondary defect. This patient is an isolated case, but she has an unaffected identical twin sister. Therefore, any identified gene mutation is probably a post-zygotic event. Additionally, the possibility of a somatic mosaic in the patient must also be considered. In any case, this is the first description of a deficiency of myotilin in a dystrophic patient, compatible with the formation of the sarcomeric structure in muscle fibers. Financial support: FAPESP-CEPID, CNPq, PRONEX.

**Mutations in the potassium channel gene KCNJ2 constitute a rare cause of long QT syndrome.** *H.M. Fodstad<sup>1</sup>, H. Swan<sup>2</sup>, M. Auberson<sup>3</sup>, I. Gautschi<sup>3</sup>, J. Loffing<sup>4</sup>, L. Schild<sup>3</sup>, K. Kontula<sup>1</sup>.* 1) Biomedicum, University of Helsinki, Finland; 2) Department of Cardiology, University of Helsinki, Finland; 3) Institute of Pharmacology and Toxicology, University of Lausanne, Switzerland; 4) Institute of Anatomy, University of Zurich, Switzerland.

Mutations of six different genes (KCNQ1, HERG, KCNE1, KCNE2, SCN5A, ankyrin-B) have been shown to cause long QT syndrome (LQTS). Andersens syndrome (AS), a rare form of periodic paralysis, associated with dysmorphic features and cardiac arrhythmias, was recently established to result from mutations of KCNJ2, a strong inward rectifying potassium channel regulating resting cell membrane potential in heart, brain and skeletal muscle. We conducted a search for KCNJ2 mutations among 185 unrelated probands with LQTS. The screening was performed by denaturing high-performance liquid chromatography and DNA sequencing. Two novel mutations of the KCNJ2 gene were detected in two families: a missense threonine to alanine mutation (T75A) in the N-terminal region (family 1) and an in-frame deletion of two amino acids (FQ163-164) in the M2 transmembrane region (family 2). In addition, a previously described silent polymorphism C1146T was detected. In family 1, some of the affected family members had a history of periodic muscle weakness characteristic of AS, but no dysmorphic features. The mean QTc interval of the affected members were 444.24 ms (family 1, n=7) and 456.8 ms (family 2, n=2). These two mutations were not found in 231 healthy control individuals. The mutations affect functionally important regions of the KCNJ2 channel protein: upon transfection of the *Xenopus* oocytes with the wild type and mutant KCNJ2 constructs, the channel proteins were correctly synthesized and addressed at the cell surface, but no measurable inward K<sup>+</sup> current could be detected for the mutant KCNJ2 constructs. In conclusion, we report two novel loss-of-function mutations of the KCNJ2 channel, affecting different domains of the channel protein. Our data also suggest that the KCNJ2 mutations found likely result in these families in a LQTS phenotype without overt manifestations of AS.

**Expression profiling reveals involvement of the GABA<sub>A</sub> receptor subunit in the fragile X syndrome. I. Gantois<sup>1</sup>, J.**

*Vandesompele<sup>2</sup>, F. Speleman<sup>2</sup>, R. D'Hooge<sup>3</sup>, L.-A. Severijnen<sup>4</sup>, R. Willemsen<sup>4</sup>, F. Tassone<sup>5</sup>, R.F. Kooy<sup>1</sup>.* 1) Dept Medical Genetics, Univ Antwerp, Antwerp, Belgium; 2) Dept Medical Genetics, Ghent University Hospital, Ghent Belgium; 3) Dept Neurochemistry and Behavior, Univ Antwerp, Antwerp, Belgium; 4) Dept Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands; 5) Dept Biological Chemistry, University of California, Davis, School of Medicine, Davis, CA.

Despite intense research it is still unclear why absence of the fragile X protein (FMRP) leads to the mental retardation, macroorchidism and specific behavior problems observed in fragile X patients. A decade of research demonstrated that FMRP, the protein missing in fragile X patients, is an RNA binding protein that shuttles between the nucleus and cytoplasm. In neurons, the protein may transport certain brain mRNAs towards the actively translating ribosomes near the synapses.

In an attempt to unravel the mechanism leading to the disorder, we performed global gene expression analysis using differential display on neurons from the fragile X mouse model, a validated model for the disorder. To verify differential expression we used microarray technology and real-time PCR. We revealed 3 differentially expressed cDNAs that showed consistent underexpression in the fragile X knockout mouse. In addition, we identified 5 genes that showed differentially expression dependent on the sample of RNA analysis, and we consider their differential expression as provisional. This list of 3 differentially expressed sequences contains a GABA<sub>A</sub> receptor subunit, a Rho guanine exchange factor 12 and an EST BU563433. It is possible that the differential expression of these genes play a role in the fragile X syndrome. E.g., absence of rho guanine exchange factor 6 causes mental retardation in patients and mice lacking the GABA<sub>A</sub> receptors subunit subunit have an increased sensitivity to epileptic seizures, like fragile X patients. Differential expression of the GABA<sub>A</sub> subunit delta receptor was shown to be restricted to the hippocampus and cortex.

**Clinical and cellular phenotype of two Italian sibs with ATLD.** *L. Chessa<sup>1</sup>, M. Piane<sup>1</sup>, S. Palmeri<sup>2</sup>, C. Savio<sup>1</sup>, G. Buscemi<sup>3</sup>, P. Lulli<sup>1</sup>, A.M.R. Taylor<sup>4</sup>, D. Delia<sup>3</sup>.* 1) Dpt. Experimental Medicine Pathology, II Faculty Medicine, Hosp S Andrea, Roma, Italy; 2) Dpt. Neurological Sciences, University of Siena, Italy; 3) Dpt. Experimental Oncology, Istituto Nazionale Tumori, Milano, Italy; 4) The University of Birmingham CRC Institute for Cancer Studies, UK.

We studied two sibs with a slowly progressive neurological syndrome mimicking ataxia telangiectasia. The neurological features appeared during infancy with unsteadiness and progressed to ataxic gait, oculomotor apraxia, nystagmus and cerebellar dysarthria. The two sibs, now 34 and 33, are still able to walk for few steps unaided. In both patients laboratory investigations, including immunoglobulins, alpha-fetoprotein, lysosomal enzymes, vitamin E and lipoproteins, resulted normal. EMG revealed a slight motor sensory neuropathy. Brain CT scan and MRI showed cerebellar atrophy with a moderate increase in the size of the fourth ventricle. Tests performed on X-irradiated peripheral blood lymphocytes at G2 phase showed mild radiosensitivity, intermediate between classical Ataxia Telangiectasia patients and controls. Western analysis revealed normal levels of ATM but markedly reduced amounts of NBS1, Mre11 and Rad50. No mutations in ATM and NBS1 genes were found. By cDNA sequence of Mre11 gene the mutated maternal allele (1442C>A) was identified, while the second allele was actually null, possibly due to nonsense-mediated mRNA decay. The sequencing of genomic DNA revealed a single base change C>T in exon 15, i.e. nucleotide 1714 in the cDNA sequence, that changes the normal codon CGA (Arg) into the stop codon TGA and reduces the Mre11 protein size from 81 to 65KDa. The patients were classified as Ataxia Telangiectasia Like Disorder (ATLD). To better understand the effects of their mutations on the function of the NMR complex we analysed the radiation induced Mre11 nuclear foci. While before irradiation ATLD cells showed a more diffuse Mre11 staining than wild-type cells, after irradiation they failed to form Mre11 nuclear foci. Moreover, the radiation induced Chk2 phosphorylation in the ATLD fibroblasts appeared defective. These results contribute to clarify the pathway of the cellular response to IR-induced DNA damage.

**AN UNUSUALLY MILD TUBEROUS SCLEROSIS PHENOTYPE ASSOCIATED WITH A MISSENSE MUTATION IN TSC2.** *M.D. D'Agostino<sup>1,2,3</sup>, A. Badhwar<sup>1,2,3</sup>, G. Gobbi<sup>3</sup>, R. Wilkinson<sup>3</sup>, D. Melanson<sup>2,3</sup>, R. Koenekoop<sup>3</sup>, M. Gans<sup>3</sup>, G. Li<sup>3</sup>, M.H. Seni<sup>1,2,3</sup>, D. Tampieri<sup>2,3</sup>, F. Andermann<sup>2,3</sup>, F. Dubeau<sup>2,3</sup>, M. Pandolfo<sup>2,4</sup>, D. Kwiatkowski<sup>5</sup>, E. Andermann<sup>1,2</sup>.* 1) Neurogenetics Unit, McGill University, Montreal, QC., Quebec, Canada; 2) Department of Neurology and Neurosurgery, Montreal Neurological Hospital and Institute, McGill University, Montreal, Quebec, Canada; 3) McGill University Hospital Centre, Montreal, Quebec, Canada; 4) Centre Hospitalier de l'Universite de Montreal, Quebec, Canada; 5) Hematology, Brigham and Womens Hospital, Boston, MA.

Tuberous sclerosis(TSC) is an autosomal dominant disorder caused by mutations in the TSC1(9q34) or TSC2(16p13) genes. The disease phenotype was found to be more severe in sporadic TSC2 patients. We analyze the genotype/phenotype correlations in a 3-generation family (202 individuals) presenting with familial epilepsy and suspicion of TSC. 58 family members were genotyped for TSC1 and TSC2 markers. We demonstrated linkage to the TSC2 locus. 28 affected individuals had a missense mutation in exon 23(2714G to A, 905R to Q). Epilepsy was diagnosed in 15/28(54%) individuals with the mutation. Seizures occurred mainly during childhood, were easily controlled, and frequently remitted spontaneously. In 24/28(86%) we detected ash-leaf or confetti spots. No facial angiofibromas, periungual fibromas or rhabdomyomata were found. Eye exams in 11/28 patients demonstrated cataract in two; glaucoma, minor intraretinal lesions, and retinal hamartoma in one individual each. Neuroimaging studies (21/28) revealed sub-ependymal giant cell astrocytoma and sub-ependymal nodules in one patient each, and hypersignal in the cerebral white matter in 7. Abdominal ultrasound detected renal angiomyolipoma in 1/14. The most frequent psychiatric diagnoses were learning disabilities (6/28) and anxiety disorder (4/28). This study expands the phenotypic spectrum of familial TSC2 and demonstrates extremely mild clinical features, in particular a benign prognosis of epilepsy. This in contrast with the more severe phenotypes described in a large series of sporadic TSC2 cases, indicating co-segregation of a modifying gene(s).

**Novel IRF6 mutations in Japanese patients with Van der Woude Syndrome: Two missense mutations (R45Q and P396S) and a 17-kb deletion.** *S. Kayano*<sup>1,2</sup>, *K. Kanno*<sup>1,2</sup>, *A. Yamada*<sup>1</sup>, *S. Kondo*<sup>3</sup>, *B.C. Schutte*<sup>3</sup>, *J.C. Murray*<sup>3</sup>, *Y. Suzuki*<sup>2</sup>, *S. Kure*<sup>2</sup>, *Y. Matsubara*<sup>2</sup>. 1) Plastic/Reconstructive Surg, Tohoku Univ Sch Medicine, Sendai Miyagi, Japan; 2) Dept. Medical Genetics, Tohoku Univ Sch Medicine, Sendai Miyagi, Japan; 3) Dept. Pediatrics, Iowa Univ, Iowa City, IA, USA.

Three Japanese families with Van der Woude syndrome (VWS) were screened for mutations in the interferon regulatory factor 6 gene (IRF6) by sequencing its entire coding region. Two novel missense mutations, R45Q in exon 3 and P396S in exon 9, were identified in families 1 and 2, respectively. In family 3, no causative base change was found by the sequencing analysis, but a deletion involving exons 4-9 was suggested by multiplex PCR analysis. To confirm the deletion and to determine its 5- and 3-boundaries, we amplified a DNA fragment containing a heterozygous polymorphic site in exon 2 by using a 5-upstream forward PCR primer and eight different reverse primers located 3 downstream of exon 2. The amplified product was subjected to nested PCR to generate a DNA fragment containing the polymorphic site. When a reverse primer located within the deletion was used for the first PCR amplification, only the non-deletion allele was detected after the second PCR. Repeated analyses with eight different reverse primers allowed us to map the boundaries of the deletion and subsequently a heterozygous 17,162-bp deletion involving exons 4-9 was identified. Since IRF6 mutations in a significant portion of VWS patients remain undetected by conventional sequencing analysis, it may be important to search for a large deletion in those patients. Our simple methods to identify deletions and to determine the boundaries of a deletion would facilitate the identification of such patients.

**Novel *de novo* mutations in *JAG1* gene.** *P.S. Lai*<sup>1</sup>, *S.L. Low*<sup>1</sup>, *W.S. Lee*<sup>2</sup>, *M. Aw*<sup>1</sup>, *S.C. Quek*<sup>1</sup>, *S.H. Quak*<sup>1</sup>. 1) Dept Pediatrics, National Univ Singapore, Singapore, Singapore; 2) Dept Pediatrics, Universiti Malaya, Malaysia.

Alagille syndrome (AGS, MIM 118450) is a multisystem developmental disorder affecting 1 in 70,000 newborns. It is characterized by bile duct paucity leading to liver disease, with involvement of other major organs. There is highly variable phenotypic expressivity, and diagnosis in mildly presenting patients can be difficult without molecular characterization. In this study, eight AGS patients of Chinese, Malay and Indian ethnic origins were identified based on histologic findings on liver biopsy with the presence of at least three of the five major clinical presenting features. Involvement of *JAG1* gene, which encodes a ligand in the Notch signalling pathway, was confirmed in all the patients. Insetion/deletion and point mutations were identified in exons 6, 10, 12, 14, 17 and 21 of *JAG1*. No deletion of the the entire *JAG1* gene was observed. All these mutations affect the evolutionarily conserved EGF-like motifs of the *JAG1* protein. One nonsense and six frameshift mutations are predicted to result in truncated protein products. A heterozygous missense mutation is expected to severely affect interactions with Notch receptor. Screening of parental genomic DNA samples indicate *de novo* origin of all the mutations. In most cases, parents of the proband did not have any clinical features consistent with a diagnosis of AGS except for one mother with features suggestive of AGS microform. Our results show a high degree of novel and *de novo* mutations occurring in the EGF-like repeats of *JAG1* in our patients.

**Novel *ENAM* mutation responsible for autosomal recessive amelogenesis imperfecta and localized enamel defects.**

*T.C. Hart*<sup>1</sup>, *P.S. Hart*<sup>2</sup>, *M.C. Gorry*<sup>1</sup>, *M.D. Michalec*<sup>2</sup>, *O. Ryu*<sup>1</sup>, *C. Uygur*<sup>3</sup>, *D. Ozdemir*<sup>3</sup>, *S. Firatli*<sup>3</sup>, *G. Aren*<sup>3</sup>, *E. Firatli*<sup>3</sup>. 1) Craniofacial Genetics, University of Pittsburgh, Pittsburgh, PA; 2) School of Public Health, University of Pittsburgh, Pittsburgh, PA; 3) School of Dentistry, University of Istanbul, Istanbul, Turkey.

The amelogenesis imperfectas (AI) are a clinically and etiologically heterogeneous group of heritable conditions characterized by qualitative or quantitative anomalies of enamel. While genetic loci have been identified for autosomal dominant and X-linked forms of AI, the genetic bases of nonsyndromic autosomal recessive forms of AI (ARAI) are unknown. The aim of this study was to evaluate five candidate genes for an etiologic role in nonsyndromic ARAI. By proband ascertainment, we identified 20 consanguineous families with AI consistent with autosomal recessive transmission. Family members were genotyped for genetic markers spanning 5 candidate genes: *AMBN* and *ENAM* (4q13.3), *TUFT1* (1q21), *MMP20* (11q22.3-q23), and *KLK4* (19q13). Genotype data were evaluated to identify loci inherited identical by descent in affected individuals. Mutational analysis was performed by genomic sequencing. Results: Homozygosity linkage studies were consistent for localization of an AI locus in three families to the chromosome 4q region containing the *ENAM* gene. *ENAM* sequence analysis in these families identified a 2 bp insertion mutation that introduced a premature stop codon in exon 10. All 3 probands were homozygous for the same g.13185\_13186insAG mutation, consistent with inheritance of the mutation from a common ancestor. These 3 probands presented with a generalized hypoplastic and undermineralized AI phenotype and a class II openbite malocclusion. All heterozygote carriers of the g.13185\_13186insAG mutation manifested localized, circumscribed hypoplastic enamel pitting defects, but none had AI nor openbite malocclusion. Conclusion: These findings demonstrate that the phenotype associated with the g.13185\_13186insAG *ENAM* mutation is dosage dependent with ARAI and openbite malocclusion segregating as a recessive trait, and enamel pitting segregating as a dominant trait.

**Identification of cryptic mutations in *IRF6* using real time deletion assays and genomic sequencing.** *N. Miwa, M. Johnson, S. Hoper, S. Kondo, T. Zuccherro, J.C. Murray, B.C. Schutte.* Pediatrics, Univ Iowa, Iowa City, IA.

We recently showed that mutations in *IRF6* cause Van der Woude syndrome (VWS). To date, we sequenced all exons and flanking intronic sequence in samples from 129 unrelated cases and identified missense (M), protein truncation (PT) and splicing (S) mutations in 84 cases (65%).

Exon	1	2	3	4	5	6	7	8	9	10
M	0	0	8	15	0	2	16	4	1	0
PT	0	1	5	6	1	9	5	2	8	0
S	0	0	1	0	0	0	0	0	0	0

There is minimal evidence for locus heterogeneity in VWS, and two large pedigrees that lack a mutation were linked to *IRF6*. Consequently, we sought evidence for point mutations that might be located outside the exons and for deletions in the 35% of samples with no mutation. We performed a real time PCR deletion assay for exons 2 and 7 on 24 samples and disclosed three likely deletions. One family appeared to be deleted at both probes. Complete genomic sequence of the 23 kb gene identified 67 SNPs. In addition, six mutations were identified in single individuals but were not identified in 90 control samples. In summary the missing mutations in VWS appear to be caused in part by the presence of genomic deletions of as yet indeterminate size and occasional mutations located within regulatory regions outside the exons. No evidence for locus heterogeneity has yet been established. These findings will provide improved genetic counseling and a better understanding of functional elements for *IRF6*.

**RFNG: a candidate gene in Russel-Silver syndrome.** *M. Ulijamperl<sup>1</sup>, D. Galron<sup>2</sup>, T. Harel<sup>1</sup>, U. Bar-yosef<sup>1</sup>, R. Ofir<sup>1</sup>, O.S. Birk<sup>1,2</sup>.* 1) Molecular Genetics, Ben-Gurion University, Beer-Sheva, Israel; 2) Soroka University Medical Center, Beer-Sheva, Israel.

Russel-Silver syndrome (RSS, OMIM #180860) occurs sporadically and is characterized by primordial growth failure, normal head circumference, a triangular face, right-left asymmetry, and fifth finger clinodactyly with normal intelligence. Approximately 10% of SRS cases show maternal uniparental disomy for chromosome 7. Less than 5% of SRS cases are caused by defects in the GRB10 gene located on chromosome 7. However, the genetic defect underlying most cases of this syndrome is unknown.

There have been two separate reports of RSS appearing in conjunction with a chromosomal breakpoint in the long arm of chromosome 17 at band 17q25, suggesting that this locus might harbor the gene defective in this disorder. Radical fringe (RFNG) is an interesting candidate gene for RSS that resides at this precise chromosomal location. RFNG is similar to *Drosophila* fringe (*fng*), which is known to participate in the evolutionarily conserved Notch receptor signaling pathway. Fringe genes encode pioneer secretory proteins with weak similarity to glycosyltransferases. Both expression patterns and functional studies support an important role for Fringe genes in patterning during embryonic development and an association with cellular transformation. The potential relevance to RSS stems from three experiments: a. fruitflies lacking functional *fng* have short legs. B. Notch has been implicated in right-left asymmetry in *C. elegans*. C. *fng* normally activates Notch by glycosylating it. Thus, in the absence of active RFNG, one would expect a phenotype of short stature and possible asymmetry, two of the hallmarks of RSS.

We have amplified by PCR and sequenced the 8 exons of RFNG and their flanking intronic regions. Thus far, of 8 RSS patients studied, none exhibited RFNG mutations. Analysis of additional RSS patients is underway.

**Mutation in GPIb impairs assembly of the platelet von Willebrand factor receptor and causes Bernard-Soulier syndrome.** *J. Tang<sup>1</sup>, S. Stern-Nezer<sup>1</sup>, P. Liu<sup>1</sup>, L. Matyakhina<sup>2</sup>, N. Luban<sup>3</sup>, S.G. Kaler<sup>1</sup>.* 1) Unit on Pediatric Genetics, LCG, NICHD/NIH, Bethesda, MD; 2) Section on Genetics and Endocrinology, DEB, NICHD/NIH, Bethesda, MD; 3) Children's National Medical Center, Washington, DC.

Haploinsufficiency for contiguous genes on chromosome 22q11 is associated with velocardiofacial (VCF) syndrome. Glycoprotein Ib (GPIb), a component of the platelet membrane receptor for the von Willebrand factor (vWF), lies within the 22q11 critical region. Isolated deficiency or impaired function of GPIb causes the bleeding disorder Bernard-Soulier syndrome (BSS) in which the platelet vWF receptor complex (GPIb-GPIb-GPIX) does not properly assemble at the platelet cell surface. Consequences range from troublesome bleeding episodes to serious hemorrhage. Treatment approaches include transfusions, desmopressin (DDAVP) to shorten bleeding time, recombinant activated factor VII, and bone marrow transplant. We identified and characterized a novel GPIb mutation in a patient with VCF and BSS. FISH in transformed lymphoblasts revealed a 22q11 deletion in the proband and neither parent. Flow cytometry showed absence of platelet surface GPIb and GPIX in the proband, and reduced levels in the patient's father. Proband DNA sequence showed normal GPIb and GPIX, and a CT transition in the GPIb coding region, predicting a proline to serine substitution (P96S) in a carboxy-terminus cystine loop. The mutation destroys a BanI site, enabling screening of other family members and normal controls. As expected from platelet flow data, the father was heterozygous for P96S. Fifty normal chromosomes did not harbor the mutation. We characterized P96S GPIb by expression experiments in a cell line (CHOIX) that stably surface-expresses human GPIb and GPIX. Flow cytometry and confocal imaging of transfected CHOIX cells demonstrated that P96S GPIb entirely abrogates surface assembly of the platelet vWF receptor complex. Based on these and other data, we propose a model of GPIb protein structure. Our results shed light on the critical importance of P96 for non-covalent association of GPIb with GPIX. Since estimated carrier frequency is 1:500, BSS may represent an under-appreciated co-morbid feature of VCFS.

**Reciprocal crossovers and a positional preference for strand exchange in recombination events resulting in deletion/duplication 17p11.2.** *W. Bi<sup>1</sup>, C.J. Shaw<sup>1</sup>, M.A. Withers<sup>1</sup>, S.-S. Park<sup>1</sup>, P.I. Pragnal<sup>1, 3</sup>, J.R. Lupski<sup>1, 2, 4</sup>.* 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Pediatrics; 3) Neurology; 4) Texas Children Hospital, Houston, Texas, 77030, USA.

Smith-Magenis syndrome (SMS) is caused by an ~3.7 Mb heterozygous interstitial deletion in 17p11.2 in about 80-90% of patients. The SMS common deletion interval is flanked by large (~200 kb), complex, and highly homologous (~98%) low-copy repeat (LCR) gene clusters-the proximal and distal SMS-REPs. These LCRs act as substrates for nonallelic homologous recombination (NAHR) resulting in both the deletion associated with SMS and the reciprocal duplication-dup(17)(p11.2p11.2). What potentially stimulates the recombination events and whether or not strand exchanges are distributed evenly throughout the regions of homology are presently unknown. Using restriction enzyme cis-morphism analyses and direct sequencing we mapped the regions of strand exchange in 16 hybrids that harbor only the recombinant SMS-REP. Our analyses showed that the sites of strand exchanges distribute widely throughout the region of homology. However, 50% of the recombinant junctions occurred in an ~12 kb region within the *KER* gene cluster of the SMS-REP. Through DNA sequencing of this hotspot, the sites of crossing-over in 7 recombinant SMS-REPs were precisely located in an ~8 kb interval. Interestingly, 4 occur in a 1.7 kb region rich with polymorphic nucleotides that could potentially reflect frequent gene conversion. For further evaluation of the strand exchange frequency in patients, novel junction fragments from the recombinant SMS-REPs have been identified in both SMS and dup(17)(p11.2p11.2) patients as predicted by the reciprocal recombination model. Putative recombination promoting sequences human minisatellite core sequence and -like sequence were identified within the hotspot. Interestingly, there is an ~2.1 kb AT-rich inverted repeat flanking the proximal and middle *KER* gene clusters but not the distal one. We hypothesize that this inverted repeat structure may stimulate double-strand breaks around this positional recombination hotspot and facilitate the unequal crossing-over.

**Rapid screening of large genomic rearrangements in the cystic fibrosis transmembrane conductance regulator (CFTR) gene by quantitative multiplex polymerase chain reaction of short fluorescent fragments (QMPSF)**

**analysis: high detection rate and diverse mutational mechanisms.** *C. Férec<sup>1</sup>, J.M. Chen<sup>1</sup>, O. Raguénès<sup>1</sup>, N.A.*

*Chuzhanova<sup>2</sup>, K. Giteau<sup>1</sup>, S. El Mouatassim<sup>3</sup>, C. Le Maréchal<sup>1</sup>, I. Quéré<sup>1</sup>, M.P. Audrézet<sup>1</sup>.* 1) INSERM 01 15, Génétique Moléculaire et Génétique Epidémiologique, Université de Bretagne Occidentale, Etablissement Français du Sang-Bretagne, Centre Hospitalier Universitaire de Morvan, Brest 29220, France; 2) Department of Computer Science, Cardiff University, Cardiff, United Kingdom; 3) Laboratoire Marcel Mérieux, Service de Génétique Humaine, Lyon, France.

Cystic fibrosis, the most common life-shortening autosomal recessive disorder in Caucasians, is caused by mutations in the CFTR gene. Despite the extensive and enduring effort of many CF researchers worldwide over the past 14 years, 1 to 30% of the disease alleles still remain to be identified. Although it has long been suggested that gross genomic rearrangements may account for these unidentified alleles, only several simple large deletions have been found by Southern blotting; and only three of them have been fully characterized. Here we reported the first systematic screening of the 27 exons of the CFTR gene for large genomic rearrangements, using the simple and rapid QMPSF technique. We analysed a well-characterized cohort of 39 patients: all had classical CF and carried at least one unidentified allele after extensive and complete screening of the CFTR gene by our well-established DGGE and DHPLC techniques. A total of 6 novel mutations including 2 simple large deletions and 4 complex large indel mutations were found, accounting for about 16% of the previously uncharacterized CF chromosomes. This represents a further significant step in understanding the molecular pathogenesis of the disease. Moreover, that all these mutations were fully characterized (ie, the breakpoints were determined) enabled us to investigate in detail their underlying mechanisms and, in particular, to trace the origin and follow the formation of the inserted sequences in the 4 complex indel mutations. This has revealed amazing new aspects of the diverse mechanisms underlying large genomic rearrangements that cause human disease.

**Fine mapping of balanced translocation breakpoints in 4 patients with non-syndromic mental retardation. L.**

*Crisponi<sup>1</sup>, S. Rassu<sup>2</sup>, C. Floris<sup>1</sup>, L. Boccone<sup>3</sup>, D. Gasperini<sup>3</sup>, G. Pilloni<sup>3</sup>, A. Cao<sup>1</sup>, G. Pilia<sup>1</sup>.* 1) Istituto di Neurogenetica e Neurofarmacologia (INN), CNR, Cagliari, Italy; 2) Università degli Studi di Cagliari, Cagliari, Italy; 3) Ospedale Regionale per le Microcitemie Cagliari, Italy.

Mental Retardation (MR) is a common disorder affecting about 3% of the human population. While several X-linked non-syndromic mental retardation loci and genes have been identified, few autosomal loci have been mapped so far. We collected 4 patients, 3 males and 1 female, with non-syndromic mental retardation associated with balanced translocations. With cytogenetic analysis we positioned the corresponding breakpoints as follow: t(X;3)(Xq13.1; 3p22.3); t(5;8)(5q14.3; 8q23.3); t(6;8)(6q14; 8q23.3); and t(7;9)(7q11.2; 9q33.3). We used Fluorescent In Situ Hybridization (FISH) analysis with Bacterial Artificial Chromosomes (BACs) as probes to map the exact position of the breakpoints in order to identify the genes affected by the translocations. In the t(X;3) patient we have been able to identify BAC clones containing the breakpoints on both chromosomes. The genomic sequence analysis of the DNA surrounding the breaks indicated that no gene had been interrupted. However, 4 candidate genes map on the 150 kb region around the Xq13 breakpoint. On 3p22.3 there is only one candidate gene about 100kb downstream to the break. For the t(5;8) and t(6;8) translocations we focused on the 8q23.3 region which contains both breakpoints. FISH analysis revealed that the two breakpoints lie 4 Mb apart. Several potential genes map in the genomic DNA around the breakpoints. It is notable that this region has been involved in the Langer Giedion Syndrome (tricho-rhino-phalangeal syndrome, type II, MIM150230) whose affected patients show mental retardation associated with other typical malformations. The MR patient with t(7;9) have been studied only in its 9q33.3 breakpoint and we mapped it in a 500 kb region. Mutation analysis in non-syndromic MR patients, functional studies, and the creation of animals with selective inactivation of candidate genes mapping at these breakpoints could lead to the identification of new genes involved in MR.

**Multiplex Ligation-Dependent Probe Amplification (MLPA): A promising technology in the detection of subtelomeric rearrangements.** *P. Hilbert, M-N. Michel, D. Sartenaer, S. Rombout, B. Parmentier, Y. Gillerot, L. Van Maldergem.* Human Genetics Center, Inst Pathologie et Genetique, Loverval, Belgium.

Unbalanced subtelomeric rearrangements can now be considered as a major cause of mental retardation and /or malformation syndrome since they are identified in up to 10 % of selected populations of mentally retarded patients . If some of the rearrangements result in a specific phenotype easily recognised by clinicians and confirmed by a single FISH analysis, the majority of subtelomeric defects produce an aspecific phenotype and require a general subtelomeres screen to be identified. Different methods of screening are available (Multiplex FISH, polymorphic markers, microarray CGH, ..) but the majority of them present important technical and /or financial limitations. Recently, two new technologies , Multiplex Amplifiable Probe Hybridisation (MAPH) and Multiplex Ligation-Dependent Probe Amplification (MLPA) were developed . They allow a rapid and inexpensive screen of all the subtelomeres from a few nanograms of DNA. The MLPA being commercially available as a kit ([www.mrc-holland.com](http://www.mrc-holland.com)) we decided to evaluate the method by testing a panel of normal patients as well as a serie of positive cases previously ascertained by cytogenetics and /or FISH in our centre. On a total of 24 rearrangements, we were able to confirm 11 duplications (probes 3 qter, 7 qter, 9 pter, 19 pter, 19 qter, 21 pter, 21q21, 21 qter, Xq11, X pter and X qter) and 11 deletions (probes 2 qter, 6 qter, 9 pter, 9 qter, 18 pter, 21 pter, 21q21, X qter, Y pter, Y q11, Y qter). Two rearrangements were missed : a 9 qter deletion and a 6 pter deletion. False positive results were also obtained with the 6 pter probe. In conclusion, our results suggest that the MLPA technology represents an interesting method, usefull in the subtelomeric rearrangements screening of mentally retarded and/or malformed patients.

**Single-sided haplotype conservation in BCM patients suggests the activation of a cryptic mutant opsin gene copy through unequal homologous recombination.** *B. Wissinger*<sup>1</sup>, *S. Tippmann*<sup>1</sup>, *J. Nathans*<sup>2</sup>. 1) Molecular Genetics Laboratory, University Eye Hospital, Tuebingen, Germany; 2) HHMI, Johns Hopkins University School of Medicine.

Blue Cone Monochromacy (BCM) is a rare X-linked visual disorder. It results from the simultaneous functional loss of middle (green) and long wavelength sensitive (red) cone photoreceptors due to mutations in the opsin gene cluster on Xq28. In normal subjects the opsin gene cluster is composed of a single red cone opsin gene and one up to five copies of the green cone opsin gene. In the majority of BCM patients there is only a single red-green hybrid opsin gene left that harbours a common missense mutation, C203R. In order to investigate this particular genotype we performed marker analysis and comparative sequencing of the opsin gene cluster in 33 independent Caucasian BCM patients. Marker analysis revealed a striking difference in haplotype conservation between flanking centromeric and telomeric chromosomal segments. There were mostly unique haplotypes centromeric to the opsin gene cluster while there were only a few common haplotypes on the telomeric side. Indeed 20 patients share a common telomeric haplotype. This difference in haplotype segment conservation is reflected also within the locus itself. Comparative sequencing of the opsin gene(s) in the BCM patients revealed high sequence variability proximal to the C203R mutation but mostly conserved sequences distal to it. This indicates that the formation of single hybrid genes carrying the C203R mutation have occurred multiple times. Analysis of 500 unselected male controls revealed a frequency of ~ 1.5% for the C203R mutation in the Caucasian population. In all controls the mutated gene copy occupies the most telomeric position within the gene cluster and thus has no or only a mild phenotypic effect (cryptic mutant allele). Intriguingly these controls show telomeric haplotype segments identical to those seen in the BCM patients. We therefore argue that the formation of the most common BCM genotype occurs through unequal homologous recombination involving a frequent founder chromosome harbouring a cryptic mutant allele.

**HOMOZYGOUS MYOTONIC DYSTROPHY CLINICAL EVALUATION, MOLECULAR ANALYSES AND GENETIC COUNSELING.** *N. Lannoy<sup>1</sup>, I. Abinet<sup>1</sup>, M. Freund<sup>1</sup>, C. Hubinont<sup>2</sup>, P. Van Den Berghe<sup>3</sup>, K. Dahan<sup>1</sup>, Ch. Verellen-Dumoulin<sup>1</sup>.* 1) Dept Genetics, UCL , Saint-Luc Hospital, Brussels, Belgium; 2) Dept of Obstetrics and gynecology, UCL, Saint-Luc Hospital, Brussels, Belgium; 3) Dept of Neurology, UCL, Saint-Luc Hospital, Brussels, Belgium.

Myotonic dystrophy (DM) is the most common autosomal dominant form of adult muscular dystrophy caused by an expansion of CTG repeat in the 3'UTR of a protein kinase gene. In the literature, 6 homozygous DM patients have been described with a phenotype similar than usually reported in heterozygotes. The purpose is to report the clinical and molecular studies of 3 additional MD homozygotes from 2 non consanguineous Belgian families and the apparent stability of intergenerational transmission. In the first family, the homozygotes are 2 sisters (aged 21 and 28) with two expanded alleles (respectively 108-607 repeats and 108-178 repeats). These 2 individuals belong to large families with a maternal and paternal history of DM. In the second family, the propositus is an 68 year old female who has bilateral cataract at 43 years of age as the only symptom of DM. The CTG expansion analyses show the presence of an apparently homozygous expanded allele (128 repeats) without normal allele. The description of DM homozygous cases in country with low consanguinity rate may be useful for clinical purposes in particular for genetic counseling and prenatal diagnosis. Furthermore, these presentation raises the question of a real appreciation of DM prevalence in Belgium.

**Deletions of the pseudoautosomal region encompassing the SHOX gene in Leri-Weill dyschondrosteosis. A.R.**

*Zinn<sup>1</sup>, B. Luo<sup>1</sup>, J.L. Ross<sup>2</sup>.* 1) McDermott Center for Human Growth and Development, The University of Texas Southwestern Medical School, Dallas, TX; 2) Department of Pediatrics, Thomas Jefferson University, Philadelphia, PA.

Leri-Weill dyschondrosteosis is characterized by short stature, mesomelia, and Madelung deformity of the wrist. Heterozygous deletions of the pseudoautosomal gene SHOX are the most frequent cause of Leri Weill dyschondrosteosis and imply that haploinsufficiency is the genetic mechanism underlying this disorder. The pseudoautosomal region (PAR1) encompassing SHOX may be prone to undergo deletions because of its high frequency of recombination during male meiosis and its abundance of repetitive DNA sequences. In order to better understand the nature of SHOX deletions, we are characterizing PAR1 microdeletions in subjects with dyschondrosteosis using a combination of FISH and somatic cell hybrids. We have examined 14 subjects with normal karyotypes (in some cases inferred from the karyotype of an affected relative) and a SHOX deletion confirmed by FISH with cosmids LLNCOYC03-M34F5 or LLNCOYC03-M15D10. Twelve of the 14 deletions break within PAR1, as judged by FISH with PAC clone RPCI3-431I1 near the proximal PAR1 boundary, whereas two of the deletions extend beyond PAR1. Thus most SHOX microdeletions lie entirely within PAR1, consistent with the hypothesized propensity of sequences within this region to undergo deletions. Because few other robust FISH probes for PAR1 sequences are readily available, we are using human/rodent somatic cell hybrids retaining the deleted X chromosome to refine the mapping of deletions. Preliminary results using PCR assays for the PPP2R3B gene distal to SHOX and the ANT3 gene proximal to SHOX demonstrate that both terminal and interstitial PAR1 deletions occur. Further characterization of these deletions will reveal whether low-copy repeat sequences in PAR1 create hotspots for SHOX deletions, as is the case for other chromosomal deletions such as Prader-Willi/Angelman and DiGeorge syndromes.

**Autosomal dominant Pterygium of the conjunctiva: a report of an India family.** *R. Meda<sup>1</sup>, R. Singh<sup>2</sup>, R.M. Memon<sup>3</sup>, J.V. Solanki<sup>4</sup>, U. Ratnamala<sup>1</sup>, U. Radhakrishna<sup>1</sup>.* 1) Genetics Laboratory, Green Cross Blood Bank & Genetic Centre, Paldi; 2) Dr. P.L. Desai Eye Center, Navrangpura; 3) Department of Zoology, Gujarat University, Ahmedabad; 4) Department of Animal Genetics & Breeding, Veterinary college, Gujarat Agriculture University, Anand, India.

The conjunctiva is the thin, transparent tissue that covers the outer surface of the eye. It begins at the outer edge of the cornea, covering the visible part of the sclera, and lining the inside of the eyelids. It also secretes oils and mucous that moistens and lubricates the eye. Pterygium, a wing-like growth of the conjunctiva is most common in people who live in tropical climates or spend a long time in sunny, windy or dusty climates. In most cases it develops late in life and in rare instances at birth. The growth of pterygium usually remains dormant; however, in some cases it grows over the central cornea and blocks light entering the eye. It also affects the vision by altering the shape of the cornea. Environmental factors may influence the penetrance. Small families with autosomal dominant modes of inheritance have been reported but no mutation-causing gene has yet been identified. We have studied a large four generation Indian pedigree with Pterygium of Conjunctiva (OMIM 178000) in which the anomaly segregates as an autosomal dominant trait. The pedigree consists of 21 individuals including seven affecteds (five males and two females). All affecteds had bilateral pterygia. The age of onset ranged from 30-35 years. Surgical excision was done in three affecteds. There was 100% penetrance in this family. The family did not have a history of long exposure to solar radiation, dust or wind, and they live in a pollution free environment. To the best of our knowledge this is only hereditary Pterygium reported with male to male transmission and provides further evidence that genetic factors make a few individuals susceptible to Pterygium of Conjunctiva. Email: [u\\_c\\_rao@hotmail.com](mailto:u_c_rao@hotmail.com).

**Linkage analysis of polycystic kidney disease in Persian cats.** *L.A. Lyons<sup>1</sup>, A.E. Young<sup>1</sup>, H.R. Roberts<sup>2</sup>, D.S. Biller<sup>3</sup>.*  
1) Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA; 2) Sierra College, 5000 Rocklin Road, Rocklin, CA 95677; 3) Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506.

Autosomal dominant polycystic kidney disease (ADPKD) is a common inherited disorder (1/1000) in humans that is characterized by the formation of fluid-filled cysts in the kidneys. Defects in the PKD1 and PKD2 genes cause 85% and 15% of human ADPKD cases, respectively. Mutations in a single gene, PKHD1, are responsible for autosomal recessive PKD (ARPKD). Mutations in several genes, including the recently described gene Nck8, cause PKD in mice. Although a number of cases of feline PKD have been described, a causative gene has not been identified. PKD affects approximately 38% of Persian cats worldwide. Forty-two microsatellites were chosen from the feline linkage and radiation hybrid maps based on known homology with human chromosomal regions that contain the PKD1, PKD2, PKHD1 and Nck8 genes. Through the use of seven extended Persian cat pedigrees, a linkage analysis has shown strong linkage, with a significant LOD score ( $Z = 5.83, = 0$ ), between a marker on feline chromosome E3, which is within 10 cM of the PKD1 gene, and the PKD disease phenotype. Further investigation into the cause of PKD will be valuable for feline health as well as provide insights into human ADPKD. The PKD1 gene is now being scanned for causative mutations.

**Characterization of early onset of retinal degeneration in Persian cats.** *H. Rah*<sup>1</sup>, *D.J. Maggs*<sup>2</sup>, *T. Blankenship*<sup>3</sup>, *L.A. Lyons*<sup>1</sup>. 1) Population Health & Reproduction, School of Veterinary Medicine, University of California Davis, Davis, CA; 2) Surgical and Radiological Sciences, School of Veterinary Medicine, University of California Davis, Davis, CA; 3) Cell Biology and Human Anatomy, School of Medicine, University of California Davis, Davis, CA.

A rapid, complete, early-onset retinopathy has been noted in Persian cats. To date, only end-stage inherited retinal degeneration (15 weeks of age) has been described. Our pedigree suggests an autosomal recessive mode of inheritance. We have characterized the inheritance, clinical, histologic, and genetic features of this disease. Kittens with known disease genotypes were produced by mating homozygous affected and heterozygous normal carriers. Seventeen cats (9 affected; 8 phenotypically normal carriers) from 4 litters were ophthalmically examined weekly from eye-opening until 16. Extent and speed of pupillary light reflexes were diminished in affected cats by 2 weeks of age and decreased further with age. Dazzle reflex was lost in affected animals by 3 weeks. Retinal vascular attenuation was first suspected at 4 weeks. Horizontal pupil diameter was greater in affected cats (7 mm) than in normal phenotype cats (4 mm) at 5 weeks. Altered tapetal reflectivity was first observed at 6 weeks of age and was generalized by 9 weeks. At 15 weeks, marked generalized retinal degeneration was seen in all affected cats. Behavioral testing confirmed total blindness in affected individuals by 16 weeks. Histological examinations suggest that photoreceptors were present and quickly degenerated between 3-15 weeks. We have identified ten genes (RPE65, RHO, PDE6B, PDE6A, RDS, RP1, CRX, CRB1, RGPR1 and GUCY2D) that we are isolating from the feline BAC library in order to screen for mutations and to isolate gene-associated microsatellite markers. Particular regions of six candidate genes with known mutations causing inheritable retinopathies in other species have been scanned. One missense mutation was identified in RP1; however, the mutation did not segregate with disease phenotype. Linkage mapping is being performed on the pedigree. We have described an autosomal recessive, rod cone dysplasia of Persian cats.

**Molecular analysis of the dysferlin gene and phenotypic spectrum of dysferlinopathies.** *K. Nguyen<sup>1</sup>, G. Bassez<sup>3</sup>, V. Labelle<sup>1</sup>, D. Figarella-Branger<sup>1</sup>, F. Leturcq<sup>2</sup>, J. Pouget<sup>1</sup>, B. Eymard<sup>3</sup>, N. Levy<sup>1</sup>.* 1) Hopital de la Timone, Marseille, France; 2) Hopital Cochin, Paris, France; 3) Hopital de la Pitie-Salpetriere, Paris, France.

Dysferlinopathies are autosomal recessive muscular dystrophies, caused by defects in the dysferlin gene. Two well-described phenotypes can result from the same defects in the dysferlin gene: limb-girdle muscular dystrophy type 2B (LGMD2B) and Miyoshi myopathy (MM). The dysferlin gene is composed of 55 coding exons in a total genomic region of 150 kb. A relatively small number of mutations was reported so far. In order to analyse gene mutations in patients with dysferlin deficiency, we performed SSCP screening of each of the 55 exons of the gene followed by sequencing. Furthermore, to investigate the phenotypic spectrum of the dysferlinopathies, we examined clinical, pathological, biological features and protein expression of 55 french patients with either LGMD2B or MM. We identified 20 novel mutations in 16 patients; one novel splicing mutation was found in three unrelated patients. We identified in majority non-sense and frameshift mutations. All these mutations were widely distributed along the coding sequence; mutational hot spots were absent. The phenotypic study revealed that 36% of the patients (from 15 families) were of maghrebian origin; 26% of the patients could not be classified as MM or LGMD2B and presented at onset with a combination of proximal and distal weakness and atrophy in the lower limbs. We introduced the term "proximodistal" for this major group. Two patients had an isolated hyperCKaemia, one of them was still asymptomatic at age 55. A minority of patients had a particularly severe progression of the disease with confinement to wheelchair in less than 5 years after onset. Misdiagnosis was frequent with diagnosis of polymyositis for 28% of the patients. The presence of inflammatory changes on muscle biopsy was significantly correlated to the severity of the disease. Finally, we established a database (Universal Dysferlin Mutations Database) in order to collect the mutational and phenotypical data and to study genotype/phenotype correlations.

Program Nr: 2298 from 2003 ASHG Annual Meeting

***MutationView* : Addition of Automatic Extraction System for Disease-associated Knowledge.** *M. Ohtsubo, S. Mitsuyama, T. Kawamura, S. Minoshima, N. Shimizu.* Department of Molecular Biology, Keio University School of Medicine, Japan.

One of the important purposes of genome research is to elucidate the genes responsible for monogenic as well as multigenic diseases. In order to better understand correlation between disease and genetic diversity, we developed the integrated database system *MutationView*, which at present mainly deals with monogenic diseases (<http://mutview.dmb.med.keio.ac.jp>). *MutationView* is the system which can search, display and analyze the mutation data with graphical environment. The characteristic features of the *MutationView* include the followings. (1) Various data display and analysis functions are available with regard to 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, case number, dominant/recessive and symptom, experimental information such as PCR primers and reaction conditions. (2) To date, we have collected 8476 entries of mutations from 1531 literatures dealing with 211 genes involved in 199 distinct diseases focused on nine categories of diseases, such as those related to ophthalmology, brain, muscle, otolaryngology, heart, syndrome, autoimmunity and familial tumor. (3) Several ways are available to access to the gene of interest through the chromosomal map, anatomical chart of disease-associated organ or tissue and diagram of causative gene product, but they are mainly gene-oriented manner. Currently, we are developing a support system to automatically extract disease-associated knowledge from the literature. Using categorized dictionary for various fields such as clinical medicine, histology and anatomy, significant relations are being extracted by statistical language analysis from OMIM.

Computer demonstration will be performed at the meeting.

**Six Families with Van Der Woude and/or Popliteal Pterygium Syndrome: All with a Mutation in the IRF6 Gene.**

*M. Ghassibe*<sup>1</sup>, *N. Revencu*<sup>2</sup>, *B. Bayet*<sup>3</sup>, *Y. Gillerot*<sup>2</sup>, *R. Vanwijck*<sup>3</sup>, *C. Verellen-Dumoulin*<sup>2</sup>, *M. Vikkula*<sup>1</sup>. 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology & Université catholique de Louvain, Brussels, Belgium; 2) Center for Human Genetics, Cliniques universitaires St-Luc and Université catholique de Louvain, Brussels, Belgium; 3) Centre Labiopalatin, Division of Plastic Surgery, Cliniques universitaires St-Luc, Brussels, Belgium.

Cleft lip with or without cleft palate (MIM#119530) (OFC) is the most frequent craniofacial malformation in humans. It results from failure of fusion of the fronto-nasal and maxillary prominences with failure of palatal shelf fusion. Its aetiology is likely multifactorial; some are a result of genetic mutations, while others may be due to environmental factors. Since 20% of OFC patients have a positive family history, genetic factors are thought to play an important role in the onset of this birth defect. Yet, the mode of inheritance of OFC remains controversial. We have initiated our genetic study of cleft lip and/or palate on syndromic OFC. We have collected 6 families with individuals affected by Van der Woude syndrome (VWS). VWS (MIM#119300) is a dominantly inherited disorder characterized by pits and/or sinuses of the lower lip, with cleft lip and/or cleft palate. It is the most common cleft syndrome. Recently, the Interferon regulatory factor-6 (IRF6) gene, localized to the VWS1 locus on 1q32.2, was shown to harbor mutations in patients with van der Woude and/or popliteal pterygium syndrome (PPS, MIM 119500). In this study, we analysed six novel VWS families, with or without PPS, for possible mutations in the IRF6 gene. Screening revealed a novel heterozygous substitution in each family. The mutations affected either the DNA-binding domain, or the protein-binding domain of the transcription factor. This data confirms that mutations in the IRF6 gene are responsible for VWS and PPS syndromes, and suggests IRF6 to be the major causative gene. (vikula@bchm.ucl.ac.be) (<http://www.icp.ucl.ac.be>).

**A further Locus for Congenital Central Hypoventilation Syndrome (CCHS).** *S. Mc Cabe*<sup>1,2</sup>, *L. Doherty*<sup>3</sup>, *W.T. McNicholas*<sup>3</sup>, *A.J. Green*<sup>1,2</sup>, *S. Ennis*<sup>1,2</sup>. 1) Department of Medical Genetics / Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Crumlin, Dublin 12, Ireland; 2) National Centre for Medical Genetics, Our Ladys Hospital for Sick Children, Crumlin, Dublin 12, Ireland; 3) Department of Respiratory Medicine / Conway Institute of Biomolecular and Biomedical Research, St Vincents Hospital, Dublin 4, Ireland.

Congenital central hypoventilation syndrome (CCHS) also known as Ondines curse, is a rare disorder of unknown etiology characterised by the failure of autonomic control of ventilation. CCHS has been associated with the neurocristopathies neuroblastoma, ganglioneuroma, and most frequently Hirschsprung disease (HSCR) which appears in 16-50% of CCHS patients, thought to be due to defective neural crest cell migration or differentiation. We describe a family, with a clinical diagnosis of CCHS comprised of a mildly affected mother, an affected father, four affected siblings, two male and two female and one unaffected female sibling, none of whom has Hirschsprung disease. Approximately 200 patients worldwide have been diagnosed with CCHS, with most reported cases being sporadic. Family cases are rare and to our knowledge this is the largest affected family reported to date and the only other case of familial recurrence without the association of HSCR. Analysis of candidate genes for Hirschsprung disease (HSCR) was carried out. Both dHPLC mutational analysis and sequencing of the candidate genes RET and GDNF together with linkage analysis of RET GDNF, EDN3, EDNRB, BDNF, SOX10, ECE1 and NRTN showed no evidence of association with the disease. The more recently described hASH1 gene on chromosome 12 (de Pontual et al) and the PhoX2b gene on chromosome 4 reported to be involved in CHSS (Amiel et al), have also been excluded in this family indicating a further disease locus for CCHS in this family. *E. J. Hum. Genet.* 2002; 10 suppl 1 :61. *Nat. Genet.* 33: 459-460, 2003.

**Exclusion of two regional candidates in MFS2, the second gene implicated in Marfan syndrome.** *C. Boileau<sup>1,3</sup>, A. Derré<sup>1</sup>, G. Jondeau<sup>2</sup>, G. Delorme<sup>2</sup>, O. Dubourg<sup>2</sup>, J-P. Bourdarias<sup>2</sup>, C. Junien<sup>1,3</sup>, G. Collod-Beroud<sup>1,4</sup>.* 1) Hôpital Necker-Enfants Malades, INSERM U383, Paris, France; 2) Service de Cardiologie and; 3) Laboratoire central de Biochimie, dHormonologie et de Génétique moléculaire, Hôpital Ambroise Paré, Boulogne, France; 4) Laboratoire de Génétique moléculaire, IURC, Montpellier, France.

Marfan syndrome is an autosomal dominant disorder presenting with genetic heterogeneity. *FBN1*, the first gene implicated in this syndrome, accounts for 80% of cases and encodes fibrillin-1, the major component of extracellular microfibrils. *MFS2*, the second gene implicated, is still unknown. It has been located in a region of less than 0.5 Mb mapped at 3p24.2-p25 between markers D3S2336 and D3S2466. The transcriptional map of the critical region contains only 2 known genes that are poor candidates (*RAR*, and *TOP2B*). We have been studying the 2 mRNAs coding for human homologs of genes known in other species and located in the region: FLJ11005 (encoding N-glycanase 1, *NGLY1*) and FLJ20604 (similar to beta-ketoacyl synthase). The expression of these candidates observed in a range of 13 different tissues is consistent with the features observed in MFS. Large and small rearrangements, point mutations as well as qualitative or quantitative transcript abnormalities were investigated. No abnormality was found excluding implication of these two genes in MFS. We are currently studying 3 predicted genes (gene models reconstructed solely from mRNA and EST evidence) in order to identify the *MFS2* gene.

**Atypical Friedreich ataxia in a carrier of a premutation length allele.** *S.I. Bidichandani*<sup>1,2</sup>, *M. Gomez*<sup>1</sup>, *S. Li*<sup>2</sup>, *T. Ashizawa*<sup>3</sup>. 1) Dept Biochem/Mol Biol, and; 2) Pediatrics, Oklahoma Univ Hlth Sci Ctr, OK; 3) UTMB Galveston, TX.

Friedreich ataxia (FRDA), the most common recessive ataxia, is caused by abnormal expansions of a GAA triplet-repeat sequence in intron 1 of the *FRDA* gene. This repeat is highly polymorphic; normal alleles contain 5-30 triplets and disease-causing alleles contain 100-1700 triplet-repeats. The carrier frequency in the Indo-European population is approximately 0.5%, consequently the vast majority of patients inherit fully expanded alleles with 600-1200 triplet-repeats. Rare alleles with 30-65 triplet-repeats, termed premutations, do not normally cause disease, but may expand into the pathogenic range when transmitted from parent to child. Equally rare intermediate alleles, ranging in size from 66-100 triplet-repeats, may be associated with mild disease. We describe a Caucasian patient (OK99) with clinical features compatible with atypical Friedreich ataxia who had one fully expanded allele (696 triplet-repeats) and another allele in the premutation size range (44 triplet-repeats). Most patients with FRDA have onset of disease at <25 y, however, approximately 15% of patients have late-onset or very late-onset Friedreich ataxia (LOFA or VLOFA), depending on whether they develop disease between 26-39 y or >40 y, respectively. OK99 first experienced difficulty with her balance at age 43. Five years after the onset of symptoms OK99 had mild, mainly cerebellar ataxia, dysarthria, hyperreflexia, loss of vibration sense, intact position sense, and slight scoliosis. The clinical severity in FRDA is mainly regulated by the length of the shorter of the two expanded alleles, and indeed most patients with VLOFA have at least one allele with <300 triplet-repeats. OK99 was tested and found to be negative for frataxin and aprataxin point mutations. Repeat expansions responsible for autosomal dominant ataxias (SCA1, 2, 3, 6, 7) were excluded, and OK99 had a normal karyotype (46,XX). These data indicate that carriers of premutation length alleles may themselves develop clinical disease in their lifetime. This study blurs the distinction between premutation and disease-causing alleles and complicates genetic counseling and phenotypic prediction in FRDA.

**Update of the FBN1 mutation database and creation of a FBN1 polymorphism database.** *G. Collod-Beroud*<sup>1,2</sup>, *I. Dehaupas*<sup>3</sup>, *S. Grey*<sup>3</sup>, *G. Matyas*<sup>4</sup>, *C. Junien*<sup>2,3</sup>, *B. Steinmann*<sup>4</sup>, *M. Claustres*<sup>1</sup>, *C. Beroud*<sup>1,2</sup>, *C. Boileau*<sup>2,3</sup>. 1) Laboratoire de Génétique moléculaire, IURC, Montpellier, France; 2) Hôpital Necker-Enfants Malades, INSERM U383, Paris, France; 3) Laboratoire central de Biochimie, dHormonologie et de Génétique moléculaire, Hôpital Ambroise Paré, Boulogne, France; 4) Division of Metabolism and Molecular Pediatrics, University Children's Hospital, Zurich, Switzerland.

We have created, in 1995, a human FBN1 mutation database, UMD-FBN1. Mutations in the FBN1 gene (encoding fibrillin-1, the major structural component of microfibrils) were first associated with Marfan syndrome (MFS) and subsequently with a large spectrum of related disorders. Aim of this database is to facilitate the mutational analysis of FBN1 mutations and the identification of structure/function and phenotype/genotype relationships. It gives access to a software package that provides specific routines and optimized multicriteria research and sorting tools. For each mutation, information is provided at gene, protein, and clinical levels. The current update shows 600 entries, of which 463 are new ones. UMD-FBN1 is accessible at <http://www.umd.be/>. The global molecular analysis reveals 2 classes of mutations. The first one, (38.6%), corresponds to mutations predicted to result in shortened fibrillin-1 molecules. They act as dominant negative but display a highly variable clinical phenotype, of which severity is directly related to the quantitative expression of the mutant allele and to the percentage of truncated or shortened proteins incorporated in the microfibrils. The second one (60.3%) correspond to missense mutations, among them mostly are located in cbEGF-like modules (78%). They can be subclassified in (a) mutations creating or substituting cysteine residues potentially implicated in disulfide bonding and consequently in the correct folding of the monomer and (b) amino acids implicated in  $Ca^{2+}$  binding and subsequently in interdomain linkage, rigidification of monomer and in protease susceptibility. Recently, we have also developed a FBN1 polymorphism database in order to facilitate diagnostics. This database list 84 reported or contributed polymorphisms.

Program Nr: 2304 from 2003 ASHG Annual Meeting

**More than 125 *FOXL2* mutations and variants in BPES and POF patients in the Human *FOXL2* Allelic Variant Database.** *D. Beysen, J. Vandesompele, F. Pattyn, A. De Paepe, L. Messiaen, E. De Baere.* Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium.

Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES, MIM 110100) is an autosomal dominant genetic condition in which an eyelid malformation is associated (type I) or not (type II) with premature ovarian failure (POF). In 2001, mutations in the *FOXL2* gene, encoding a forkhead transcription factor, have been shown to cause BPES type I and II. Since then a number of publications appeared describing *FOXL2* mutations in BPES patients. In addition, there have been reported a few *FOXL2* variants in POF patients and XX males. Previously, our group has reported the existence of two mutational hotspots in *FOXL2* and of intra- and interfamilial phenotypic variability in BPES families. Moreover, we have demonstrated genotype-phenotype correlations for a number of mutations in BPES patients. Here we describe a new locus-specific Human *FOXL2* Allelic Variant Database (<http://allserv.ugent.be/~jvdesomp/foxl2/>), created using the MuStaR software (on which the *PAX6*, *PAX2*, *SHOX* and *MLYCD* Allelic Variant Databases have been based similarly). Our database contains general information about the *FOXL2* gene, as well as details about more than 125 intragenic mutations and variants of *FOXL2*, obtained from published papers and abstracts of meetings, and also from unpublished data of our group. Not included in the current version of the database are complete *FOXL2* deletions, microdeletions and cytogenetic rearrangements of the *FOXL2* region on 3q23. The aim of this database is to provide an online resource, allowing remote users to do queries by selecting options on a web form and to submit new mutations to the database by means of a submission form. We believe this database will be a useful tool as it contains prevalence data about disease-causing mutations and a catalogue of polymorphisms. In addition, it will also allow to make more accurate genotype-phenotype correlations.

**Huntingtin and huntingtin-associated protein 1 influence neuronal calcium signaling mediated by inositol-(1,4,5) triphosphate receptor type 1.** *E.Y. Chan<sup>3</sup>, T. Tang<sup>1</sup>, H. Tu<sup>1</sup>, A. Maximov<sup>2</sup>, Z. Wang<sup>1</sup>, C.L. Wellington<sup>4</sup>, M.R. Hayden<sup>3</sup>, I. Bezprozvanny<sup>1</sup>.* 1) Department of Physiology and; 2) Center for Basic Neuroscience, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas, USA; 3) Center for Molecular Medicine and Therapeutics, Department of Medical Genetics Children's and Women's Hospital and; 4) Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada.

Calcium (Ca<sup>2+</sup>) has long been regarded to play a critical role in the pathogenesis of Huntington's disease (HD). Dysfunctional neuronal Ca<sup>2+</sup> homeostasis caused by Huntingtin (Htt) with polyglutamine expansion (exp) has been previously described to involve ionotropic glutamate receptors (e.g. NMDA receptors) and the mitochondria. The type 1 inositol (1,4,5)-triphosphate receptor (InsP3R1) is an intracellular Ca<sup>2+</sup> release channel that plays an important role in neuronal function. In a yeast two-hybrid screen with the InsP3R1 carboxy terminus, we isolated Htt-associated protein-1A (HAP1A). We have demonstrated that an InsP3R1-HAP1A-Htt ternary complex is formed *in vitro* and *in vivo*. InsP3R1 activation by InsP3 *in vitro* is sensitized by Httexp, but not normal Htt. Transfection of full-length Httexp or caspase-resistant Httexp, but not normal Htt, into medium spiny striatal neurons facilitates Ca<sup>2+</sup> release in response to threshold concentrations of the selective mGluR1/5 agonist 3,5-DHPG. Our findings identify a novel molecular link between Htt and InsP3R1-mediated neuronal Ca<sup>2+</sup> signaling and provide an explanation for the derangement of cytosolic Ca<sup>2+</sup> signaling in HD patients and mouse models.

**Nonsense mediated decay mitigates the effect of *MPZ* mutant alleles responsible for a more severe peripheral neuropathies.** *M. Khajavi*<sup>1</sup>, *T. Oyama*<sup>1</sup>, *K. Inoue*<sup>1</sup>, *J.R. Lupski*<sup>1,2</sup>. 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Dept Pediatrics, Texas Children's Hospital, Houston, TX.

Charcot-Marie-Tooth disease (CMT) is a clinically and genetically heterogenous group of inherited neuropathies that is traditionally classified into two major types, CMT type 1 (CMT1) and CMT type 2 (CMT2). In most CMT1 patients (~70%) the disease phenotype results from a 1.5-Mb DNA duplication on chromosome 17p11.2-p12 (CMT1A) whereas other patients have mutations in one of several genes such as peripheral myelin protein 22 (*PMP22*), myelin protein zero (*MPZ*), connexin 32 (*Cx32/GJB1*), early growth response 2 (*EGR2*), periaxin, and neurofilament light chain gene. Protein Po, encoded by the *MPZ* gene, is the major structural protein of peripheral myelin and functions as a homophilic adhesion molecule in myelin compaction. Mutations in the *MPZ* gene cause dominantly inherited peripheral neuropathies with various severity from CMT disease (mild alleles) to Dejerine-Sottas syndrome/congenital hypomyelinating neuropathy (severe alleles). Various nonsense and frameshift mutations in *MPZ* result in both mild and severe forms of peripheral myelinopathy, but no functional consequences have been delineated to effectively explain the association between mutation and disease severity. Interestingly, most mutations in *MPZ* that results in PTCs in downstream exons in which the nonsense-mediated decay (NMD) pathway is insensitive, appear to result in more severe forms of peripheral neuropathy. Such severe disease-associated alleles do not retain the transmembrane domain, suggesting that the toxic effects of these mutant proteins do not require positioning in the plasma membrane where the normal tetrameric *MPZ* proteins localize and function. Considering the location of truncation in the gene, here we investigated whether mutations that could possibly trigger the NMD RNA surveillance pathway and therefore decrease mRNA levels can decrease disease severity. To address this, we have introduced such disease associated mutations in an expression vector and used a transiently transfected HeLa cell lines to study the potential role for NMD in the abrogation of aberrant effect of selected mutant alleles.

**Molecular genetic study of patients with BOR Syndrome: Current status of mutation analysis and issue of genetic heterogeneity.** *S. Kumar<sup>1</sup>, M. Hare<sup>1</sup>, B. Berrigan<sup>1</sup>, C.W.R.J. Cremers<sup>2</sup>, W.J. Kimberling<sup>1</sup>*. 1) Dept Genetics, Boys Town Natl Research Hosp, Omaha, NE; 2) Department of Otolaryngology, University Hospital Nijmegen, The Netherlands.

Branchio-oto renal syndrome (BOR) is a human developmental disorder characterized by (1) branchial cleft, fistulas or cysts (2) various ear anomalies such as malformed pinnae, pre-auricular pits, structural defects of the external, middle and/or inner ear (3) hearing loss and (4) renal anomalies. It is inherited as an autosomal dominant pattern and affect at least 2% of profoundly deaf children and have estimated prevalence of 1 in 40,000. The phenotypic expression of the BOR gene is extremely variable from one family to another. The first BOR gene has been localized to chromosome 8q13 and has been shown to be caused by mutations in the EYA1 gene. A second genetic locus associated with BOR syndrome has been localized to chromosome 1q. We have performed mutation analysis on more than eighty five BOR families by WAVE followed by sequence analysis of sixteen EYA1 exons. To date, using this approach, we have identified nineteen novel mutations. At least 55% of our large data set of BOR families do not show mutations in the EYA1 gene. This further complicates the issue and suggests that either most of the mutations lie in the untranslated region or several genes are involved in the branchiogenic disorder. We have performed genetic linkage analysis on several multigenerational BOR type families with 8q and 1q markers to explore the possibility of further genetic heterogeneity. The present results, together with mutation screening and genetic linkage study, demonstrate genetic heterogeneity. A genome-wide search is being performed for the localization of third locus. Also, a candidate gene analysis is underway to identify genes in the critical region of second BOR locus on chromosome 1q. These results provide the basis for a molecular genetic testing and genetic counseling of members of BOR families. Further characterization of EYA1 mutation and identification of other BOR genes will significantly help in defining the spectrum of defects associated with branchial and hearing anomalies.

**The molecular basis of classical Ehlers-Danlos Syndrome (EDS).** *A. de Paepe, F. Malfait, S. Symoens, B. Loeys, L. Nuytinck, P. Coucke.* Depart. Medical Genetics, 0 K5, University Hospital Gent, Ghent, Belgium.

The classical form of EDS is characterized by fragile and hyperextensible skin, poor wound healing, atrophic scarring and joint hypermobility. Mutations in the COL5A1 and the COL5A2 gene encoding the 1 and 2 chain of type V collagen have been shown to cause the disorder, but it is unknown which proportion of classical EDS patients carries a mutation in these genes. We have studied fibroblast cultures from 52 patients with classical EDS for the presence of type V collagen defects. First, a biochemical analysis was performed by SDS-PAGE of metabolically labeled collagens produced by dermal fibroblasts. An abnormal collagen pattern was detected in only 4/52 cell lines, making this method not conducive for diagnostic evaluation. Secondly, a test that permits us to detect the presence of a non-functional COL5A1 allele (null-allele) was developed. In 36% of informative patients a null-allele was found (15/42). Finally molecular analysis of the COL5A1/A2 gene was performed by SSCP, CSGE and DHPLC. Six structural mutations in the COL5A1 gene and 3 in the COL5A2 gene were detected. In addition 10 mutations, either nonsense, frameshift or splice site mutations, all leading to a premature termination codon, were identified. Overall, the presence of a molecular defect in type V collagen could be confirmed in 26/52 patients (50%) with classical EDS. Variability in severity of the phenotype was observed. Two patients, harbouring exonskipping mutations in the COL5A1 and the COL5A2 gene respectively, had a particularly severe phenotype with bilateral hip dislocation. The relatively low mutation detection rate, despite exhaustive mutation analysis, suggests that other genes are involved in classical EDS. This is validated by our findings of a COL1A1 mutation (Arg134Cys) in 2 unrelated patients with the classical EDS phenotype. In addition, mutations in Tenascin X have been demonstrated to cause an autosomal recessive condition similar to classical EDS. Studies in transgenic mice have suggested genes encoding small leucine-rich proteoglycans (SLRP) as candidate genes for EDS. However no human examples have been documented for these mouse models at present.

**Refinement of the ichthyosis-prematurity locus on chromosome 9q34.** *M. Larsson<sup>1</sup>, J. Klar<sup>1</sup>, M. Pigg<sup>1</sup>, B. Carlsson<sup>1</sup>, A. Vahlqvist<sup>2</sup>, T. Gedde-Dahl JR.<sup>3</sup>, N. Dahl<sup>1</sup>.* 1) Dept Genetics & Pathology, Uppsala Univ, Uppsala, Sweden; 2) Dept Medical Science, Uppsala Univ, Uppsala, Sweden; 3) Institute of Forensic Medicine, Rikshospitalet, Oslo, Norway.

Autosomal recessive congenital ichthyosis (ARCI) is a heterogeneous group of skin disorders. Ichthyosis-Prematurity Syndrome (IPS), is a rare form of ARCI with a relatively high prevalence in the Norwegian population. Key features are thick caseous desquamating epidermis and complicated pregnancies due to polyhydramnion and an opaque amnion fluid caused by the shedding of large amount of epidermally derived cells. This results in premature birth of the affected child.

We recently reported the mapping of a novel IPS locus in thirteen families related to a defined region in middle-Norway and Sweden. The results showed a maximum cumulative lod-score of 3.73 on chromosome 9q34.

Here we present five additional families and the refinement of the critical interval using a denser map of microsatellite markers and SNPs. Haplotype analysis resulted in the identification of two separate ancestral haplotypes associated with the disease. The minimal shared haplotype region spans a 21-kb region containing a single gene.

**Acquired Pseudoxanthoma Elasticum-like syndrome in  $\alpha$ -Thalassemia Patients.** *O. Le Saux*<sup>1</sup>, *N. Hamlin*<sup>1</sup>, *K. Beck*<sup>1</sup>, *B. Bacchelli*<sup>2</sup>, *P. Cianciulli*<sup>3</sup>, *I. Pasquali-Ronchetti*<sup>2</sup>. 1) Pacific Biomed Research Ctr, University of Hawaii, Honolulu, HI; 2) Dept Biomed Sci, University of Modena and Reggio Emilia, Modena, Italy; 3) S. Eugenio Hospital, Rome, Italy.

A marked frequency of patients diagnosed with  $\alpha$ -thalassemia develop clinical and histopathologic manifestations of a disease called pseudoxanthoma elasticum (PXE). These manifestations include the calcification of elastic fibers in the skin, eyes, and cardiovascular system. PXE is also an inherited disorder, however, it is caused by mutations in an ATP-binding cassette gene called ABCC6 localized on the short arm of chromosome 16. Haplotype analysis of the ABCC6 locus in Italian  $\alpha$ -thalassemia/PXE patients and their family members revealed no co-segregation with the PXE phenotype. Moreover, SSCP analysis and direct sequencing were used to screen all 31 exons of the ABCC6 gene in 10 patients presenting both  $\alpha$ -thalassemia and PXE symptoms. This mutational analysis did not detect any of the 81 disease-causing mutations known thus far. Furthermore, an RT-PCR showed the expression of ABCC6, in skin fibroblasts from a  $\alpha$ -thalassemia/PXE patient, was similar to that of control individuals. These results revealed that the PXE phenotype present in  $\alpha$ -thalassemia/PXE patients is not likely caused by known mutations of ABCC6, suggesting that some  $\alpha$ -thalassemia patients have a susceptibility to developing an acquired form of PXE as a consequence of their altered metabolism.

**Genome Scans Provide Evidence for Keloid Susceptibility Loci on Chromosomes 2q23 and 7p11. E.**

*Reichenberger*<sup>1</sup>, *A. Marneros*<sup>2</sup>, *J.E.C. Norris*<sup>3</sup>, *S. Watanabe*<sup>4</sup>, *B.R. Olsen*<sup>2</sup>. 1) Dept BioStructure & Function, Univ Connecticut Health Ctr, Farmington, CT; 2) Dept Cell Biology, Harvard Medical School, Boston; 3) St.Luke's/Roosevelt Hospital Center, New York; 4) Dept of Plastic and Reconst. Surgery Showa University School of MedicineTokyo, Japan.

Keloids are proliferative fibrous growths that result from an excessive tissue response to skin trauma. They often occur sporadically, but in some families a genetic predisposition to keloids has been observed. Here we studied two families with an autosomal dominant inheritance pattern of keloids. One African-American family showed a high degree of variability in the extent of keloid formation between family members, whereas the second family from Japan showed a pattern of full penetrance and formation of only small keloids. We performed a genome wide linkage search for genes predisposing to keloid formation in these two families. We identified linkage to chromosome 2q23 (maximal two-point LOD-score 3.01) for the Japanese family from Japan. The African-American family showed evidence for a keloid susceptibility locus on chromosome 7p11 (maximal two-point LOD score of 3.16). The observed locus heterogeneity in autosomal dominant keloid disease is consistent with the clinical heterogeneity of this wound healing disorder. Dense microsatellite analysis in these two loci was performed and candidate genes were identified. This study provides the first genetic evidence for keloid susceptibility loci and serves as a starting point for the identification of responsible genes.

**A Novel Homozygous Recessive Mutation in the Galactosyltransferase-I (B4GALT7) Gene in Individuals Resembling the Progeroid Type of Ehlers-Danlos Syndrome.** *M.F. Ul Haque<sup>1</sup>, A.S. Teebi<sup>1,3</sup>, M. Al-Ali<sup>2</sup>, M.S. Al-Mureikhi<sup>2</sup>, S. Kennedy<sup>1,3</sup>, G. Al-Thani<sup>2</sup>, S.H.E. Zaidi<sup>1</sup>, L-C. Tsui<sup>1,4</sup>.* 1) Dept Genetics, Elm Wing, Hosp Sick Children, Toronto, ON, Canada; 2) Dept Pediat, Hamad Med Corp, Doha, Qatar; 3) Clin Genet and Dymorp, Hosp Sick Children, Toronto, ON, Canada; 4) Univ of Hong Kong, Pokfulam Rd, Hong Kong.

Ehlers-Danlos syndrome (EDS) comprised of a heterogeneous group of inheritable disorders that exhibit skin hyperextensibility, joint hypermobility and tissue fragility. In addition to these classical features of EDS, the Progeroid type displays wrinkled loose facial skin, fine curly hair, and scanty eyebrows and eyelashes. Several genes have been implicated to produce EDS phenotypes. In the present study, two individuals from two sibships of a large consanguineous family from Qatar exhibiting the phenotype that resembles the Progeroid type of EDS, were analyzed for linkage to the genes that have been implicated in EDS. Homozygosity was observed for only two markers on chromosome 5q35.2 that are associated with the B4GALT7 gene, in which compound heterozygous mutations have previously been reported in a single case of Progeroid-like EDS. The entire coding region and exon/intron boundaries of B4GALT7 gene were sequenced and a missense, C to T substitution at nucleotide 808 in the coding region was discovered in the DNA from both affected individuals. While this mutation was not found in control subjects of same ethnicity, carrier parents were heterozygous for this change. Pedigree analysis further confirmed the recessive mode of inheritance in this type of EDS. The C to T substitution translates into an arginine to cysteine change at the amino acid residue 270 of galactosyltransferase-I (encoded by the B4GALT7 gene). This change in the catalytically active, extracellular, C-terminal domain is likely to produce an abnormal protein due to misfolding or aberrant interaction with other extracellular proteins. The resulting abnormal protein product could alter the normal organization of extracellular matrix of connective tissues where the galactosyltransferase-I is expressed, thus conferring the Progeroid like phenotype in affected subjects.

**Mutations of the RNA-specific adenosine deaminase gene (*DSRAD*) are involved in Dyschromatosis Symmetrica Hereditaria.** T. Suzuki, Y. Miyamura, K. Inagaki, M. Kono, S. Ito, N. Suzuki, Y. Tomita. Department of Dermatology, Nagoya University School of Medicine, Nagoya, Aichi, JAPAN.

Dyschromatosis symmetrica hereditaria (DSH, reticulate acropigmentation of Dohi) has been reported mainly in Japan, although it occurs in families of every ethnic origin all over the world. The patients have pinpoint, pea-sized hyper- and hypo-pigmented macules on the back of the hands and the top of the feet. These abnormalities are otherwise asymptomatic and do not affect the general health. DSH generally shows an autosomal dominant pattern of inheritance with high penetrance. Many clinical and morphological investigations have been reported, but the cause and the pathogenesis as well as the *DSH* gene have not yet been clarified. Accordingly, we mapped and positionally cloned the *DSH* gene. We performed a genome-wide search in three families (39 affected and 41 unaffected), and mapped the *DSH* locus to chromosome 1q21.3. To further refine the localization, we identified novel single nucleotide polymorphisms and integrated the genetic and physical maps of the region. The final genetic interval was approximately 500 kb in which 9 genes were mapped. To detect the pathological mutations, affected individuals from each of the Pedigrees 1, 2 and 3, plus new pedigree, named Pedigree 4, were screened by PCR-SSCP and direct sequencing. The results showed that they were heterozygous for mutation alleles of double-strand RNA-specific adenosine deaminase gene (*DSRAD*), R474X, L923P, K952X and F1165S respectively. None of these four mutations were found in 55 unaffected individuals in any of the four pedigrees or in 116 unrelated normally pigmented Japanese adults that were surveyed. Thus, we concluded that those four mutations are not polymorphic but are the pathologic ones causing the disease. *DSRAD* catalyzes the deamination of adenosine to inosine in double-stranded RNA substrates which results in the creation of alternative splicing sites. We speculate that the failure of correct RNA editing may induce differentiation of melanoblasts to hyper- or hypo-active melanocytes colonizing in an irregular distribution in the skin lesions during the development.

**Frequency of Mitochondrial DNA (T/C) 16189 Polymorphism among Filipinos with Type 2 Diabetes Mellitus. E.**

*Paz-Pacheco*<sup>2</sup>, *E. Cutiongco*<sup>1</sup>, *C. Halili-Manabat*<sup>3</sup>, *M.A. Lim-Abrahan*<sup>2</sup>, *C. Padilla*<sup>1</sup>, *K.D.S. Corvera*<sup>2</sup>. 1) Institute of Human Genetics, National Institutes of Health, University of the Philippines, Manila; 2) Section of Endocrinology, Diabetes, and Metabolism, Department of Medicine, College of Medicine, University of the Philippines Manila; 3) Department of Physiology, College of Medicine, University of the Philippines Manila.

Mitochondrial DNA polymorphisms, implicated in the development of type 2 diabetes mellitus, have a reported prevalence that is higher among Asian diabetics than their normal counterparts. To determine the frequencies of mitochondrial DNA (T/C) 16189 polymorphism among Filipino type 2 diabetic patients and normal controls, we extracted DNA from peripheral blood obtained from 31 type 2 diabetic patients and 31 normal controls. We subjected these samples to polymerase chain reaction, restriction enzyme digestion, and gel electrophoresis techniques. Preliminary analysis of DNA was performed. Among the 29 diabetics, 14 presented with the wild type allele, while 4 had the (T/C) 16189 polymorphism. Interestingly, 17 of the 38 study subjects showed a restriction enzyme digestion pattern different from either the wild type or the (T/C) 16189 polymorphism. Type 2 diabetics who possess the said polymorphism had lower body weights, body mass indices, and abdominal circumferences, but had higher mean arterial pressures than the type 2 diabetics with the wild type allele. The wild type allele was present in 48.3 percent of the type 2 diabetics as compared to 22.22 percent of the controls. Only 4 of the 29 diabetics (13.8 percent) and 1 of the 9 controls (11.1 percent) had the (T/C) 16189 polymorphism. Different restriction enzyme digestion patterns were found in 44.8 percent of the study population. Additional molecular studies need to be performed among the latter group of subjects to further elucidate these observed variations.

**Mutation spectrum at the ALADIN locus in Allgrove syndrome: founder mutation and neurodegenerative phenotypes.** *A. Tullio-Pelet, D. Trochet, F. Begeot, A. Munnich, S. Lyonnet.* INSERM U393 Dept Genetique, Hopital Necker Enfants Malades, Paris, France.

Triple-A syndrome (Allgrove syndrome, MIM 231550) is a rare autosomal recessive disorder characterized by adrenal insufficiency with adrenocorticotropin hormone (ACTH) resistance, achalasia of the oesophageal cardia and alacrima. Several lines of evidence indicate that triple-A syndrome results from the abnormal development of the nervous system including late-onset progressive neurological symptoms such as peripheral neuropathies and anisocoria. Moreover, the central nervous system may also be involved as cerebellar ataxia and mild dementia have been reported. In 2000, we showed that mutations of the *AAAS* gene, encoding a nucleoporin protein (ALADIN), was disease causing in triple A syndrome. We have screened the 16 exons of *AAAS* gene in 49 patients using DHPLC and direct DNA sequencing. In 42 patients (86%) we have identified the causing mutation (missense, nonsense, insertion or deletion). Among them, 35/42 (83%) patients were true homozygous and 24/35 carried the IVS14+1G A founder mutation, characterized in Northern African population. Only 7 patients were compound heterozygous. In the remaining 7 patients (14%), no mutation could be found ; among these, the diagnosis of triple A syndrome was regarded as unlikely for 4 cases. These data suggest genetic homogeneity in Allgrove syndrome. They support a Mediterranean founder effect for mutation IVS14+1G A that extended to France and to Puerto Rican population. Finally, both expression studies and this study underline the involvement of the *AAAS* gene in the central and peripheral nervous system.

**PABPN1 (GCN) expansion mutations in OPMD are likely due to unequal crossovers during recombination. B.**

*Brais<sup>1</sup>, Q.D. Trinh<sup>1</sup>, H. Lavoie<sup>1</sup>, A. Saint-Denis<sup>1</sup>, C. Alexander<sup>1</sup>, F.M.S. Tomé<sup>2</sup>, M. Fardeau<sup>2</sup>, B. Eymard<sup>2</sup>, A. Pou Serradell<sup>3</sup>, M. Medici<sup>4</sup>, C. Camejo<sup>4</sup>, M. Rodriguez<sup>4</sup>, J. Mathieu<sup>5</sup>, J.P. Bouchard<sup>6</sup>, G.A. Rouleau<sup>7</sup>.* 1) CHUM, Université de Montréal, Qc, Canada; 2) Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, France; 3) Hospital Universitari del Mar, Barcelona, Spain; 4) Hospital de Clínicas, Montevideo, Uruguay; 5) Carrefour de Santé, Jonquière, Qc, Canada; 6) Hôpital de l'Enfant-Jésus, Quebec, Qc, Canada; 7) McGill University Health Center, Montreal, Qc, Canada.

Autosomal dominant oculopharyngeal muscular dystrophy (OPMD, OMIM 164300) causes late-onset progressive eyelids ptosis and dysphagia. It is particularly frequent in French Canadians (FC). It is caused by (GCN) expansions in the PABPN1 gene (14q11.1). Slippages during replication and unequal crossovers during recombination have been proposed as mutation mechanisms. Purpose. Determine what is the likely mechanism responsible for the genesis of OPMD mutations. Methods. We sequenced PABPN1 mutations in 66 cases from 49 ethnic backgrounds. We constructed haplotypes for in 42 cases with the shared (GCG)<sub>9</sub> FC mutation, four FC cases in which the size of the mutation has increased and 62 carrier chromosomes from 54 ethnic backgrounds. Results. The lengthening of the (GCG) repeat causes OPMD in 80% of families. In the other 20%, cryptic addition of combinations of (GCA) and (GCG) codons are the causal mutations. Lengthening of the (GCG) repeat was uncovered in the four FC cases with larger size mutations. The ancestral FC haplotype was lost, likely through unequal recombinations, on one side of the mutation in at least 3 of the 4 families. Haplotype clustering of cases from different ethnic backgrounds identified three groups of at least three families sharing telomeric or centromeric haplotypes. In these groups, different sizes of mutations were observed and two included cryptic mutations. Conclusion. This study demonstrates that 20% of OPMD PABPN1 mutations are due to cryptic (GCN) mutations. Together our results suggest that unequal crossovers during recombination are responsible for the appearance and subsequent expansions of (GCN) PABPN1 mutations in OPMD.

**Screening for mutations in the connexin 31 gene in Brazilian patients with non-syndromic deafness. E.L.**

*Sartorato<sup>1</sup>, F. Alexandrino<sup>1</sup>, C.A. Oliveira<sup>1</sup>, A.T. Maciel-Guerra<sup>2</sup>.* 1) Lab de Genetica Humana/CBMEG, Universidade Estadual de Campinas, SP, Brasil; 2) Departamento de Genetica Medica/FCM, Universidade Estadual de Campinas, SP, Brasil.

Deafness is a complex disorder that involves a high number of genes and environmental factors. At this moment, there has been enormous progress in non-syndromic deafness research, with the identification of 90 loci and 33 nuclear and 2 mitochondrial genes. Mutations in the GJB3 gene encoding the gap junction protein connexin 31 (Cx31) have been pathogenically linked to erythrokeratoderma variabilis and non-syndromic autosomal recessive or dominant hereditary hearing impairment. To determine the contribution of connexin 31 to sporadic deafness, we analysed the entire gene of connexin 31 in 67 families with non-syndromic hearing impairment and no mutation in the connexin 26 gene. Single coding exon of connexin 31 was amplified from genomic DNA and then sequenced. We reported three amino acid changes, Y177D, 49delK and R32W, and two nucleotides variants which represents a silent mutation. The R32W substitution has been previously described, and its involvement in hearing impairment remains uncertain. We presume that mutations in connexin 31 gene are an infrequent cause of non syndromic deafness.

Program Nr: 2318 from 2003 ASHG Annual Meeting

**Detailed analysis of the unstable region of the ZNF9 gene causing DM2/PROMM in normal chromosomes.** *M. Baiget, L. Martorell, I. Cusco, M. Calaf.* Genetics Department, Hosp Santa Creu i Sant Pau, Barcelona, Spain.

It has been recently demonstrated that large expansions in the intron 1 of the ZNF9 gene, located on chromosome 3q21.3 cause myotonic dystrophy type2 (DM2/PROMM). The aim of this study is to analyze the structure/size of the unstable repeat track containing the CCTG sequence in normal chromosomes. We have studied 30 unrelated alleles. DNA was amplified as previously described (Liquori et al 2001). PCR products were cloned using a commercial kit (Invitrogen) and subsequently sequenced. To investigate the pattern of inheritance of the unstable sequence, we have also studied two transmissions of the repeat sequence in a control family. The PCR products from the members of this family were analyzed using FAM as a fluorescent dye and processed in an ABI Prism 310 (Gene Scan software, Perkin Elmer-Applied Biosystems). In control alleles, the (CCTG)<sub>n</sub> ranged from 7 to 22, the CCTG13 being the most frequently observed. All tracks except one were interrupted by GCTG and TCTG. In control alleles, the size of the (TG)<sub>n</sub>(TCTG)<sub>n</sub>(CCTG)<sub>n</sub> repeat track ranged from 106 to 180 base pairs, 128bp, 138bp and 140 bp being the most frequently observed alleles. This complex region has been transmitted in a stable manner in the studied control family. Sequence and Gene Scan analysis demonstrate the presence of mosaicism in the (TG)<sub>n</sub>(TCTG)<sub>n</sub>(CCTG)<sub>n</sub> repeat track in somatic DNA samples from normal subjects.

**SCA8 BAC Transgenic Mice: Pathogenic CTG Expansion and Expression Models.** *M. Moseley, M. Weatherspoon, D. Jin, H.B. Clark, J.W. Day, L.P.W. Ranum.* Institute of Human Genetics, University Minnesota, Minneapolis, MN.

Spinocerebellar ataxia type 8 is caused by an untranslated CTG expansion. To better understand SCA8 pathogenesis we have created a transgenic mouse model of the disease by replacing the normal repeat tract (11 CTGs) of an SCA8-containing bacterial artificial chromosome (BAC) with a pathogenic expansion of 107 CTGs. The BAC contains approximately 115 kb of sequence flanking the SCA8 gene, which should be sufficient to regulate transgene expression in the mouse to mirror human temporal and tissue specific expression patterns. Seven expansion and 3 control lines were obtained (copy # 1-10). SCA8 expression, detected by RT-PCR in all lines, generally mimics the human expression pattern with low levels of transcripts found in the CNS. Three expansion lines had poorer rotarod performance at 26-wks than BAC control and non-transgenic littermates. Two of the highest copy number lines have a progressive, severe phenotype in which animals have difficulty in locomotion, lose weight and die (14 to >52 wks). In contrast, the five lower copy number expansion lines develop a milder, slowly progressive phenotype much later in life (~18 mos) in which an uncoordinated and unsteady gait is observed. Similar phenotypes have not been observed in BAC transgenic control lines with the normal repeat tract. No evidence of Purkinje cell or CNS neurodegeneration has been found, suggesting that the progressive neurological phenotype observed in the mice is caused by neuronal dysfunction rather than neurodegeneration. To better understand the pathology of the SCA8 transcript we are generating a second BAC transgenic expression model in which -gal reporter expression is driven by the endogenous human promoter contained on the BAC. Temporal and cell specific expression patterns of the human SCA8 promoter are being characterized and correlated with molecular and functional changes in the CNS of affected mice containing pathogenic CTG expansions. Our data provide the first evidence in a model organism that SCA8 transcripts containing an expanded repeat cause a progressive neurological phenotype, and support an RNA gain of function model of SCA8 pathogenesis.

**Myotonic Dystrophy Type 2: Founder Haplotype and Evidence for Loss of Sequence Interruptions on a Premutation Allele.** *Y. Ikeda<sup>1</sup>, C.L. Liquori<sup>1</sup>, M. Weatherspoon<sup>1</sup>, K. Ricker<sup>2</sup>, B.G.H. Schooser<sup>3</sup>, J.C. Dalton<sup>1</sup>, J.W. Day<sup>1</sup>, L.P.W. Ranum<sup>1</sup>.* 1) Inst of Human Genet, Univ of Minnesota, Minneapolis, MN; 2) Univ of Wurzburg, Germany; 3) Ludwig-Maximilians-Univ, Munich, Germany.

Myotonic dystrophy (DM), the most common form of muscular dystrophy in adults, can be caused by a mutation on either chromosome 19 (DM1) or 3 (DM2/PROMM). In 2001 we demonstrated that DM2 is caused by a CCTG expansion in intron 1 of the *zinc finger protein 9 (ZNF9)* gene. To investigate the ancestral origins of the DM2 expansion, we compared haplotypes of 71 genetically-confirmed DM2 families using 19 short tandem repeat markers we developed that flank the repeat tract. All of the families are Caucasian, with the majority of Northern European/German descent and a single family from Afghanistan. Several conserved haplotypes spanning more than 700 kb converge into a single haplotype near the repeat tract. The common interval immediately flanking the repeat in all DM2 families extends up to 216 kb telomeric and 119 kb centromeric of the CCTG expansion. Although estimates of haplotype age are difficult because rare ancestral recombination events are unlikely to be evenly distributed from generation to generation, and microsatellite mutation rates vary from marker to marker, conservation of a native Tajik Afghan haplotype allows us to speculate that DM2 was introduced into Afghan gene pool sometime between 2000 and 1000 B.C. when the ancient Aryan tribes of Indo-European extraction settled Aryana (ancient Afghanistan). The DM2 repeat tract contains the complex repeat motif  $(TG)_n(TCTG)_n(CCTG)_n$ . The CCTG portion of the repeat tract is interrupted on normal alleles, but as in other expansion disorders these interruptions are lost on affected alleles. We examined haplotypes of 228 control chromosomes and identified a potential premutation allele with an uninterrupted  $(CCTG)_{20}$  on a haplotype that was identical to the most common affected haplotype. Our data suggest that the predominant Northern European ancestry of DM2 families resulted from a common founder and that the loss of interruptions within the CCTG portion of the repeat tract may predispose alleles to further expansion.

**Expansion to full mutation of a FMR1 gray zone allele over two generations.** *A. Terracciano, M.G. Pomponi, G.M.E. Marino, G. Neri.* Institute of Medical Genetics, Catholic University, Rome, Rome, Italy.

The fragile X syndrome is due to an expansion of the CGG repeat sequence at 5' UTR of the FMR1 gene. According to size of the CGG sequence, one can distinguish four allele categories: normal (40 CGG), protomutated or grey zone (40-59 CGG), premutated (60-200 CGG) and full mutated (200 CGG). However, the boundaries among these categories are blurred, especially those of the grey zone. This uncertain classification makes it difficult to recognize truly unstable alleles and to accurately estimate the risk of expansion. We report on a family in which the proband presented with mental retardation and hyperactivity. Southern blot analysis showed a 1,2 kb methylated full mutation. PCR analysis of other members of the family showed two alleles of 29 and 61 CGG respectively repeats in the mother. Sequencing of the 61 CGG allele showed no AGG interruptions. Both mother's sisters had two alleles of 31 and 43 CGGs respectively, and the daughter of one of these had two alleles of 24 and 43 repeats, demonstrating stable transmission of the 43 CGG allele. The maternal grandmother had two alleles of 29 and 31 CGGs. The maternal grandfather had died several years before. Molecular analysis confirmed paternity with a probability of 99,97 for all three sisters. According to these findings it is likely that the maternal grandfather carried the 43 CGG allele, showing unstable transmission, because it expanded first to 61 CGGs in one daughter, and then to full mutation in her grandchild. On the other hand, the allele remained stable in three other persons we could observe (maternal aunts and cousin of the proband). Therefore, genetic counselling is difficult for female carriers in this family. Although we cannot exclude paternal mosaicism, it is likely that a rare event of progression from protomutation to premutation occurred in this family .

**Mapping genes for Autosomal Dominant Keratoconus.** *B.A Bejjani*<sup>1,2</sup>, *M. Gajecka*<sup>1</sup>, *A. Molinari*<sup>3</sup>, *J. Pitarque*<sup>3</sup>, *S.M. Leal*<sup>4</sup>, *R.A. Lewis*<sup>4</sup>. 1) Wash State Univ, Spokane; 2) Sacred Heart Med Ctr, Spokane; 3) Hospital Metropolitano, Quito, Ecuador; 4) Baylor Col Medicine, Houston, TX.

Keratoconus (KC) is a non-inflammatory thinning and anterior protrusion of the cornea that results in steepening and distortion of the cornea, altered refractive powers and altered visual acuity. The cause(s) of KC are unknown. We identified an Ecuadorian population among whom isolated KC seems to segregate in an autosomal dominant trait with reduced penetrance. We have examined, collected blood, and purified DNA from 104 individuals from 28 multiplex families with KC. We excluded *VSX1* as a candidate for KC in these families by sequence analysis of *VSX1*-coding exons in 18 KC individuals. We identified three single nucleotide polymorphisms (SNPs) in the *VSX1* coding region: 18GT, 174GT and 542AG. These are presumed to be benign SNPs because none induces a putative amino-acid change in the sequence of *VSX1*, and two of them (18GT and 542AG) were found in matched normal control samples sequenced in parallel. We are confirming these results with targeted genotyping with markers at the *VSX1* locus on 20p11q11. Additional markers on chromosome 16q22.3-q23.1 are being examined to evaluate a KC locus there described in a Finnish population. To evaluate whether the pedigree data have sufficient power to detect linkage, we performed a simulation study with the following assumptions: an autosomal dominant model; a polymorphic marker with 5 alleles of equal frequency (Heterozygosity = 0.8); a disease allele frequency of 0.001; and a penetrance of 0.70 for homozygous and heterozygous disease allele carriers and 0.0 for homozygous wild-type genotypes. The marker and the disease locus were generated in complete linkage ( $= 0.0$ ). The data were generated both with all families linked to one locus and under linkage heterogeneity where only a proportion of the families, , segregates the same disease locus. We used a variety of values and analyzed data with the ELODHET program. These simulation studies show that sufficient power exists to establish linkage even if only 60% of the families are linked to a single locus. A genome-wide screen is in progress.

**A novel locus for autosomal dominant hypodontia involving incisors and premolars.** *P. Das*<sup>1</sup>, *A. Rohr*<sup>1</sup>, *R.M. Scarel-Caminaga*<sup>3</sup>, *E. Aguiar*<sup>4</sup>, *S.M. Leal*<sup>2</sup>, *E.L. Figuerido*<sup>4</sup>, *S.R.P. Line*<sup>3</sup>, *P.I. Patel*<sup>1, 2</sup>. 1) Dept Neurology, Baylor Col Medicine, Houston, TX; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Faculty of Odontology of Piracicaba-UNICAMP, Brazil; 4) Federal University of Alagoas, Maceio, Brazil.

Familial hypodontia is a genetically heterogeneous condition characterized by the congenital absence of primary and/or permanent teeth, ranging in severity from mild, involving the absence of one or two teeth, to severe, where the majority of teeth can be missing. Autosomal dominant (AD) hypodontia has been localized to at least three chromosomal loci: *MSX1*, *PAX9*, and an unknown locus on chromosome 10. A missense mutation in *MSX1* has been associated with AD hypodontia involving the second premolars and third molars while frame-shift, nonsense and missense mutations in *PAX9* as well as a submicroscopic deletion involving the *PAX9* gene have been seen in families with AD hypodontia involving primarily molar teeth. A third locus associated with hypodontia involving the absence of a few to the entire set of permanent teeth has been mapped to chromosome 10q11.2. A distinct unknown locus underlying hypodontia involving incisors and premolars has been previously implicated based on exclusion mapping of candidate genes. We have identified a large family segregating AD hypodontia involving incisors and premolars, and studied 31 members of whom 15 are affected. A total genome scan assuming autosomal dominant inheritance with complete penetrance has allowed mapping of the hypodontia locus to a 1.6 cM (730 Kb) interval on chromosome 17q23. This interval contains 16 genes. Sampling of additional family members and fine mapping will allow narrowing of the candidate region. High-throughput sequencing of all candidate genes is expected to allow identification of this novel hypodontia locus. (Supported by NIH grant DE14102 and Center for Inherited Disease Research).

**Familial juvenile hyperuricaemic nephropathy (FJHN): Linkage analysis in 17 families, transcriptional characterization of the FJHN critical region on chromosome 16p11.2 and the analyzes of uromodulin and other candidate genes.** *K. Hodanova<sup>1</sup>, M. Kalbacova<sup>1</sup>, B. Stiburkova<sup>1</sup>, J. Majewski<sup>2</sup>, A. Marinaki<sup>3</sup>, A. Simmonds<sup>3</sup>, G.*

*Matthijs<sup>4</sup>, J. Fryns<sup>4</sup>, J. Ott<sup>2</sup>, S. Kmoch<sup>1</sup>.* 1) Charles University, Inst of Inher Metab Diseases, Prague, Czech Republic; 2) Laboratory of Statistical Genetics, Rockefeller University, New York, NY, USA; 3) Purine Research Unit, GKT, Guys Hospital, London, UK; 4) Center for Human Genetics, University of Leuven, Leuven, Belgium.

FJHN is an autosomal dominant renal disease characterized by juvenile onset of hyperuricaemia, gouty arthritis, and progressive renal failure at an early age. Recent studies showed linkage of a gene for FJHN to the same genomic region on chromosome 16p11.2, where the gene for the phenotypically similar medullary cystic disease type 2 (MCKD2) has been localized. In our study of 17 families we found in 8 of them linkage of FJHN to 16p11.2, which suggests that, in a large proportion of FJHN kindreds, the disease is likely to be caused by a gene(s) located outside of 16p11.2. Haplotype analysis of all analyzed families provided two non-overlapping critical regions on 16p11.2 FJHN1, delimited by markers D16S499-D16S3036 and FJHN2, delimited by markers D16S412-D16S3116. Considering MCKD2 to be a distinct molecular entity, the analysis suggested that as many as three kidney disease genes may be located in close proximity on 16p11.2. From genomic databases we compiled transcription maps of whole critical genomic region in which 122 known genes and predicted loci have been localized. We selected, analyzed and found no pathogenic mutations in ten candidate genes. Following recent publications of Uromodulin (UMOD) mutations in FJHN and MCKD2 we analyzed UMOD coding sequence including exon/intron boundaries in seven 16p11.2 linked FJHN families. FJHN causing mutations (Cys126Arg, Met229Arg, and Cys317Tyr) were found in only three of them. Further investigations are aimed at sequencing of UMOD 6 kbp promotor and large deletions detection. This effort should finally answer question of FJHN and MCKD2 genetic heterogeneity on 16p11.2.

**Two novel severe mutations in the pancreatic secretory trypsin inhibitor gene (SPINK1) cause familial or/and hereditary pancreatitis.** *C. Le Maréchal<sup>1</sup>, J.M. Chen<sup>1</sup>, C. Le Gall<sup>2</sup>, G. Plessis<sup>3</sup>, J. Chipponi<sup>4</sup>, N.A. Chuzhanova<sup>5</sup>, O. Raguénès<sup>1</sup>, C. Férec<sup>1</sup>.* 1) INSERM 01 15, Génétique Moléculaire et Génétique Epidémiologique, Etablissement Français du Sang-Bretagne, Université de Bretagne Occidentale, Centre Hospitalier Universitaire de Morvan, Brest, France; 2) Hépatogastroentérologie Nutrition, Hôpital Édouard Herriot, Lyon, France; 3) Département Génétique et Reproduction, Centre Hospitalier Universitaire de Caen, Caen, France; 4) Service de Chirurgie Générale et Digestive, Centre Hospitalier Universitaire, Clermont-Ferrand, France; 5) Department of Computer Science, Cardiff University, Cardiff, United Kingdom.

Mutations in the pancreatic secretory trypsin inhibitor (PSTI/SPINK1) gene have recently been found to be associated with chronic pancreatitis. The aim of this study was to identify novel severe SPINK1 mutations with a view to expanding the gene's mutational spectrum and providing further insights into SPINK1's role in pancreatitis. 46 unrelated families, each including at least two pancreatitis patients and carrying neither cationic trypsinogen mutations or the frequent SPINK1 N34S mutation, participated in this study. The four exons and their flanking sequences of the SPINK1 gene were screened by denaturing high performance liquid chromatography analysis; and mutations were identified by direct sequencing. A heterozygous microdeletion mutation (27delC), which occurs within a symmetric element, was identified in two families. In one family, 27delC showed segregation with the disease across two generations, with a penetrance of up to 75%. But in the other family, however, the same mutation manifested as a low-penetrance susceptibility factor. In addition, a novel heterozygous splicing mutation, IVS2+1G>A, was found in one family with familial pancreatitis. Our results demonstrated that genetic testing for SPINK1 mutations in pancreatitis families wherein no cationic trypsinogen mutations were found is warranted; and suggested that the differing views about SPINK1's role (ie, disease-causing vs. disease) in pancreatitis should be discussed in the context of specific mutations.

**Cirhin, the protein mutated in North American Indian childhood cirrhosis (NAIC) is a novel nucleolar protein.**

*B. Yu, G. Mitchell, A. Richter. Génétique Médicale, Hôpital Sainte-Justine, Montréal, Canada.*

NAIC (MIM 604901) is a severe, pediatric onset intrahepatic cholestasis, frequent in the Natives of NE Quebec. A C->T mutation in *cirhin* (NM\_032830) changes a conserved Arg to Trp at codon 565 (R565W) in patients, altering the predicted secondary structure of the 686 aa protein. As a step in the elucidation of the function of cirhin, we used bioinformatics to predict organellar localization. These showed multiple targeting signals including a bipartite nuclear localization signal (BNLS, aa 328-344) and a C-terminal nuclear localization signal (C-NLS, aa 677-686). To test the predictions we transfected EGFP and His tagged fusion constructs of wild type (wt) and R565W cirhin into HeLa cells. Our results show that both proteins locate to the nucleolus. Thus, cirhin is a nucleolar protein lacking obvious RNA and DNA binding motifs. To investigate its function within the plurifunctional nucleolus we mutated the predicted targeting signals. The C-NLS (PPPIKKKK) conforms to the consensus for monopartite NLS, a helix-breaking N-terminal proline followed 3 or more basic residues in an array of 7-9 aas. To isolate the function of the NLS we used a construct that initiates at M487. As expected, it localizes to the nucleus. Changing the second Lys to Ala (PPPIKAKK) causes loss of nuclear localization, indicating the importance of this residue for NLS function. Truncation of the C-NLS does not completely abolish nucleolar localization, but its presence improves efficiency. We have evidence of a nucleolar localization signal (NrLS) of ~170 aas (M315-M487). This includes the predicted BNLS. With truncation and mutagenesis we are defining the minimal sequence of this NrLS. The nuclear and nucleolar localization signals are functionally additive, suggesting that different transport mechanisms may be necessary for correct and efficient nucleolar targeting of cirhin. Yeast 2 hybrid studies underway may reveal the effect of other interacting proteins on targeting of cirhin to this organelle. Cirhin is a nucleolar protein with a unique NLS and an NrLS having a more complex structure than others previously described. Supported by the Canadian Institutes of Health Research.

**Recent development studies in MTMR13, the gene implicated in a demyelinating Autosomal Recessive form of Charcot-Marie-Tooth (AR-CMT) disease associated with Early Onset Glaucoma (EOG).** *H. Azzedine*<sup>1</sup>, *A. Bolino*<sup>2</sup>, *A. Chojnowski*<sup>1</sup>, *K.H. El hachimi*<sup>3</sup>, *C. Blondin*<sup>4</sup>, *P. Hamard*<sup>4</sup>, *P. Fragner*<sup>1</sup>, *G. Stevanin*<sup>1</sup>, *A. Brice*<sup>1</sup>, *E. Le guern*<sup>1</sup>. 1) INSERM U289-NEB, Hopital de la Salpetriere, Paris, France; 2) Dulbecco Telethon Institute, Laboratories of Molecular Genetics, Gaslini Institute, and Department of Paediatrics and Center of Excellence for Biomedical Research, University of Genova, Genova, Italy; 3) INSERM U106, Hopital de la Salpetriere, Paris, France; 4) Hopital des quize-vingt, sevice 3, Paris, France.

CMT is a pathological and genetic 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 on anatomopathological and electrophysiological grounds: demyelinating and axonal CMT. The genetic heterogeneity is highlighted by the various modes of inheritance (autosomal dominant, X linked and autosomal recessive forms and the expanding number of different assigned loci (n=30). In some families from Japan and Brazil, a demyelinating CMT, mainly characterized by the presence of myelin outfoldings on nerve biopsies, co-segregated as an autosomal recessive trait with EOG. We first mapped this syndrome to chromosome 11p15.3 in a 4.6 cM region overlapping the locus for an isolated demyelinating ARCMT (CMT4B2) in two large consanguineous North African families. More recently, we identified in these two families two different nonsense mutations in the Myotubularin-related 13 gene, MTMR13. The MTMR family includes proteins with a phosphoinositide phosphatase activity, and proteins in which key catalytic residues are missing, and that are thus called pseudo-phosphatases. MTMR13 may be important for the development of both the peripheral nerve and the trabeculum meshwork, which permits the outflow of the aqueous humor. Both of these tissues have the same embryonic origin. Expression studies of MTMR13 during myelination, trabecular meshwork and retina development in rat, mouse and human is in progress using quantitative real time PCR assay, Northern blots and in situ hybridisation. The finalized results will be presented during the meeting.

**Does Tyrosinase modify the Congenital Glaucoma phenotype in Saudi Arabia?** *N. Hernandez*<sup>1,2</sup>, *A. Al-Rajhi*<sup>3</sup>, *D.W. Stockton*<sup>4</sup>, *R.A. Lewis*<sup>4</sup>, *J.R. Lupski*<sup>4</sup>, *B.A. Bejjani*<sup>1,2</sup>. 1) Wash State Univ, Spokane; 2) Sacred Heart Med Ctr, Spokane; 3) King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia; 4) Baylor Col Medicine, Houston, TX.

Primary Congenital Glaucoma (PCG) is an autosomal recessive disorder for which we showed decreased penetrance in the Saudi Arabian population. Approximately 94% of individuals with PCG in Saudi Arabia have homozygous or compound heterozygous mutations in cytochrome P4501B1 (*CYP1B1*). Investigation of families with multiple affected individuals with PCG showed that penetrance is about 50%. Recently, Tyrosinase (*Tyr*) was found to be a modifier of the drainage structure phenotype in *Cyp1b1*<sup>-/-</sup> mice, with *Tyr* deficiency increasing the magnitude of dysgenesis. The severe dysgenesis in newborn mice lacking both *CYP1B1* and *TYR* was alleviated by administration of the tyrosinase product dihydroxyphenylalanine (L-dopa) to the pregnant mothers (Libby et al. *Science*:299;1578-1581). To evaluate a possible role for *TYR* in the modification of the clinical PCG phenotype in the Saudi population, we sequenced all five *TYR*-coding exons in 60 individuals with homozygous and compound heterozygous *CYP1B1* mutations. Thirty-five of those individuals were nonpenetrant and 25 had full expression of the disease. Initial analysis of our data shows that there is a heterozygous mutation in exon 4 of each of three fully penetrant individuals and six nonpenetrants. Evaluation of this mutation in 100 control Saudi chromosomes is in progress. Analysis of linkage data of markers that map to chromosome 11q14-21 does not support this locus as a potential modifier for PCG in the Saudi population.

**Germ cell selection of an FGFR2 point mutation explains the paternal age effect in Apert syndrome.** A. Goriely<sup>1</sup>, G.A.T. McVean<sup>3</sup>, M. Röjmyr<sup>4</sup>, B. Ingemarsson<sup>4</sup>, A.O.M. Wilkie<sup>1, 2</sup>. 1) Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK; 2) Nuffield Dpt of Clinical Laboratory Sciences, University of Oxford, UK; 3) Dpt of Statistics, University of Oxford, UK; 4) Pyrosequencing AB, Uppsala, Sweden.

Observed mutation rates in humans appear higher in male than in female gametes and often increase with paternal age. This bias, usually attributed to the accumulation of replication errors or inefficient repair mechanisms during spermatogenesis, has been difficult to study directly. Apert syndrome, a dominantly inherited congenital malformation (birth prevalence ~1 in 70,000) characterised by craniosynostosis and syndactyly, is caused in ~98% of cases, by *de novo* nucleotide transversions (755C>G or 758C>G) in Fibroblast Growth Factor Receptor 2 (FGFR2). These mutations, which encode FGFR2 proteins with enhanced ligand binding, occur at a rate elevated ~500-fold over background, arise exclusively from the unaffected father and show a paternal age effect.

Using Pyrosequencing technology, we have developed a robust method to quantify the levels of the 755C>G mutation (responsible for ~2/3 of Apert syndrome cases) in the sperm of healthy male donors. We show that this mutation is present at significant levels (maximum of 1 in 6,000) in the sperm of many men, but is not substantially higher in the fathers of Apert syndrome children (n=6). Although the mutation levels increase with donor age, explaining the observed paternal age effect, we show, surprisingly, that the original mutational events occur infrequently. Instead of originating from repeated replication errors that accumulate with age, we propose that these infrequent FGFR2 mutations are enriched because they confer a selective advantage to the spermatogonial stem cells in which they arise, leading to clonal expansion of the mutant germ cell population. This work has implications for diverse fields, including clinical genetics, reproductive biology, stem cells, cancer and evolution.

Program Nr: 2330 from 2003 ASHG Annual Meeting

**Identification of transcripts regulated by PITX2 in the human ocular anterior segment.** *M.A. Lines, O. Vicaruddin, M.A. Walter.* Departments of Medical Genetics and Ophthalmology, University of Alberta, Edmonton, AB, Canada.

Mutations of the paired-bicoid homeodomain transcription factor *PITX2* cause Axenfeld-Rieger (AR) malformations of the ocular anterior chamber, as well as abnormalities of the teeth, umbilicus, and heart. In approximately fifty percent of cases, patients with AR develop secondary glaucoma due to impaired uveal aqueous drainage. While several genes governed by *PITX2* have been identified in the heart and dental primordia, ocular *PITX2* target genes remain to be discovered. We are employing two complementary methods to study *PITX2*-driven gene expression in cell lines derived from the human trabecular meshwork and nonpigmented ciliary epithelium, tissues that drain and secrete aqueous humor respectively. RNA interference was used to abrogate *PITX2* activity and effect changes in target gene expression, which will be measured via cDNA microarray. Additionally, chromatin immunoprecipitation will be used to isolate *PITX2*-responsive regulatory elements upstream of target genes. Delineating the ocular regulon governed by *PITX2* will improve our understanding of the normal development and function of these tissues, while indicating genes potentially involved in the etiology and/or pathogenesis of AR.

**Structure and expression analysis of Interferon Regulatory Factor 6 (IRF6).** A.S. Knight<sup>1</sup>, N.K. Rorick<sup>2</sup>, M.I. Malik<sup>2</sup>, J.C. Murray<sup>2</sup>, M.J. Dixon<sup>1</sup>, B.C. Schutte<sup>2</sup>. 1) Dental Medicine and Surgery, Univ Manchester, UK; 2) Dept Pediatrics, Univ Iowa.

We recently discovered that mutations in IRF6 underlie two related orofacial clefting disorders, Van der Woude and popliteal pterygium syndromes. IRF6 belongs to the IRF family of transcription factors that share a highly conserved DNA binding domain and a less well conserved protein binding domain. Several members of this family use alternative splicing to produce transcripts that encode protein isoforms that negatively regulate the full-length molecule. Northern analysis of embryonic tissues showed transcripts of approximately 2.1, 4.1 (predicted) and 20 kb. Using PCR of human and mouse cDNA panels and alignment of ESTs, we did not detect any transcripts that lack exons encoding IRF6. Based on northern and EST analysis, the 2.1 and 4.1 kb transcripts are consistent with alternative polyadenylation in exon 9, and the 20 kb transcript is consistent with an unspliced transcript. Subsequently, we performed in situ hybridization and immunohistochemical experiments on a developmental series of morphologically-staged mouse embryos. We found that *Irf6* is expressed in the leading edges of the developing facial processes at E10/E11 and in the medial edge epithelia of the palatal shelves immediately prior to and during their fusion on E14.5. Immunohistochemical analysis supports the observation that *Irf6* expression is restricted to the medial edge epithelium and suggests a common function for *Irf6* in the fusion events driving development of the primary and secondary palate. *Irf6* expression was also observed in the apical ectodermal ridge of the developing limbs at E10/E11, the molar and incisor tooth germs from E12-E15, the fusion zone on the ventral aspect of the developing genitalia from E11 onwards, and in the skin, hair and vibrissae follicles from E12. These regions of expression correlate strongly with the VWS and PPS phenotypes. *Irf6* expression was also observed in the lymph nodes. As no aspect of the VWS and PPS phenotype corresponds with this expression pattern, the function of IRF6 in these tissues is likely to be compensated by other members of the IRF family.

**A giant gene encoding a developmentally regulated novel member of the M28 peptidase family is severed by a Cornelia de Lange-associated translocation breakpoint at 3q26.3.** *E. Tonkin<sup>1</sup>, M. Smith<sup>1</sup>, P. Eichhorn<sup>1</sup>, S. Jones<sup>1</sup>, B. Imamwerdi<sup>1</sup>, M. Jackson<sup>1</sup>, S. Lindsay<sup>1</sup>, N. Carter<sup>2</sup>, T.-J. Wang<sup>1</sup>, M. Ireland<sup>1</sup>, J. Burn<sup>1</sup>, I. Krantz<sup>3</sup>, T. Strachan<sup>1</sup>.* 1) Inst Human Genetics, Univ Newcastle, Newcastle, United Kingdom; 2) Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 3) Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia and The University of Pennsylvania School of Medicine, Philadelphia, PA.

Cornelia de Lange (CdLs) syndrome is a developmental malformation syndrome characterised by mental handicap, growth retardation, distinctive facial features, limb reduction defects and various other features including gastrointestinal and respiratory system abnormalities. A CdLs gene at 3q26.3 has been postulated on the basis of phenotypic overlap with trisomy 3q cases who have in common an extra copy of the 3q26-q27 region, and the occurrence in a classical CdLs patient of a de novo balanced translocation with a breakpoint at 3q26.3. We have identified the two breakpoints in this individual and show that the 3q26.3 breakpoint severs a novel giant gene containing at least 27 exons spanning 1.4 Mb. Northern blot analysis identified up to six different transcripts ranging from 1.3 kb to 10 kb in length with strongest expression in kidney and placenta. In situ hybridization studies show embryonic expression in various locations including the mesonephros and the duodenum. Transcript analysis has identified extensive alternative splicing in the 5' UTR plus variable use of coding sequences. Predicted protein isoforms fall within the 337-795 amino acid range and are defined by the use of alternative initiating methionine codons separated by 17 amino acids, and additionally by alternative C-terminal sequences generated by alternative splicing/polyadenylation. Sequence analysis of the predicted protein has identified a large C-terminal domain that is shared with the M28 family of zinc peptidases. Preliminary mutation screening of a panel of CdLs patient samples failed to identify patient-specific mutations.

**An innovative protocol combining multiplex QF-PCR and fluorescent conformation sensitive capillary electrophoresis (FCSCCE) for unified dosage analysis and point mutation screening.** *E.J. Ashton, S.C. Yau, Z. Hatton, S.J. Abbs.* Genetics Centre, Guy's Hospital, London, England.

Large multi-exon genes such as dystrophin, exhibiting heterogeneous mutation spectrums have, until now, required independent tests and sometimes different samples to screen for (1) gross deletions and duplications and (2) point mutations. This multi-method approach is cumbersome and inefficient for even medium throughput testing. Therefore we have developed a streamlined, comprehensive mutation screening protocol for DNA to detect deletions, duplications and point mutations in the dystrophin gene of both males and females. This unified method is applicable to any gene.

All 79 dystrophin exons are amplified in 12 multiplex QF-PCR assays and the products analysed on a multicapillary fluorescent analyser to detect exon copy number. The same PCR products are then subjected to FCSCCE to screen for point mutations using a multicapillary analyser with modified polymer. In a control study CSCE detected 98% of point mutations.

A blind trial of 50 male and 50 female samples is currently in progress to evaluate the sensitivity of the assay for detecting all mutation types. We have completed the analysis of the 50 male samples and have obtained 100% specificity and sensitivity. 28 large deletions/duplications were correctly identified, in 9 of these cases the end points were defined where they had not been previously. 8 substitutions were detected (including 1 which was not previously known about) and in 7 samples no mutation was detected. 6 of the 7 samples were from normal control males and the remaining sample was known to contain a deeply intronic mutation in a region not covered by this assay.

We have demonstrated the power of this method by applying it for a DMD family in which no samples were available from affected males and an intragenic recombination had left a pregnant female with up to a 50% carrier risk. A nonsense mutation was quickly identified in an obligate carrier, which was not present in the pregnant relative.

**Joubert Syndrome: a patient with a de novo t(2;22)(q13;q11.1).** *E. Hatchwell*<sup>1</sup>, *N. Tommerup*<sup>2</sup>, *U. Kristoffersson*<sup>3</sup>, *R. Stanyon*<sup>4</sup>, *S. KANTARCI*<sup>1</sup>. 1) Cold Spring Harbor Lab, Cold Spg Harbor, NY; 2) Dpt. of Medical Genetics, IMBG, The Panum Institute, University of Copenhagen, Copenhagen, Denmark; 3) Dpt. of Clinical Genetics, The University Hospital of Lund, Lund, Sweden; 4) Comparative Molecular Cytogenetics Core, NCI, Frederick, MD.

Joubert Syndrome (JS) is a rare autosomal-recessive condition involving agenesis or dysgenesis of the cerebellar vermis with accompanying brainstem malformations. JS is further characterized by hypotonia, developmental delay, intermittent hyperpnea, and abnormal eye movements. The biochemical and molecular basis of JS remains unknown. To map the translocation breakpoint in a patient with Joubert syndrome associated with a de novo, apparently balanced translocation t(2;22)(q13;q11.1), we have used flow sorted derivative chromosomes. In order to delineate the t(2;22)breakpoint, we initially generated a microarray containing BAC sets that span chromosomes 2 and 22 at roughly 1 MB intervals. We amplified the flow sorted chromosomes using degenerate oligonucleotide primer PCR (DOP-PCR) with a primer distinct in sequence to those used to generate BAC DNA for the array, in order to avoid cross-hybridization. We also used a recently developed method, known as Multiple Displacement Amplification (MDA) on our flow sorted material. In MDA, an isothermal reaction is performed at 300C, using f29 DNA polymerase, which synthesizes strands in excess of 70 kb. The major advantage of this method is the relative uniformity of locus representation. Amplified products generated from each of the flow sorts was differentially labeled using Cy3 and Cy5 and hybridized to our microarray. We narrowed the translocation breakpoint to a region between two BACs that are separated by 4MB. STS-PCR was performed at higher density levels and the breakpoint localized to within a 324 bp region. The breakpoint on chromosome 2 interrupts a predicted gene and we are currently engaged in analysis of this candidate gene in cases of JS.

**Functional analysis of dystrophin sequence variants: biological/clinical relevance.** *C.S. Richards<sup>1</sup>, L.H. Chin<sup>1</sup>, O. Lichtharge<sup>2</sup>, T. Schwede<sup>3</sup>, J. Kopp<sup>3</sup>, P.A. Ward<sup>1</sup>, G. Miller<sup>4</sup>, D.R. Love<sup>5</sup>, M.R. Hegde<sup>1</sup>.* 1) Diagnostic Sequencing Laboratory; 2) Computational Biology Laboratory, Department of Molecular and Human Genetics, Baylor College Medicine, Houston, TX; 3) Division of Bioinformatics, University of Basel, Switzerland; 4) Texas Childrens Hospital, Houston, TX, USA; 5) 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. We have developed strategy to interpret dystrophin variants using multiple approaches based on mutation-type to determine biological relevance. A comprehensive mutation analysis has been developed for the analysis entire dystrophin gene to identify whole exon deletion/duplication mutations, point mutations, small deletions and small insertions using denaturing high performance liquid chromatography (dHPLC). We have extended our studies utilizing a variety of approaches to understanding the biology of how dystrophin variants affect the structure and function of dystrophin protein in muscle and influence the expression of other genes in multiple pathways. These approaches include Evolutionary Trace to identify highly conserved and functionally important residues, protein modeling using SwissModel server and Deepview, microarray analysis to assess effects of variants on differential gene expression, and confirmation by biological studies including transcript and immunohistochemical analyses of presumptive relevant muscle proteins. Dystrophin sequence variants currently reported as unclassified variants will now be interpreted in a clinically relevant manner based on further analysis of dystrophin protein structure and function, and will provide useful information about genotype and phenotype relationships. We present here data on a single patient with a mutation in splicing mutation in intron 3 (exon3+1G>C), previously not documented. We plan to extend our efforts in future collaborative work to develop therapeutic approaches, for patients in whom we have characterized clinically relevant non-classical-type mutations.

**Genotype-Phenotype Correlation of Recessive Hereditary Inclusion Body Myopathy (IBM2).** *D. Darvish, S. Naiem, Y. Huo.* H.I.B.M. Research Group, Inc., Encino, CA.

Recessive hereditary Inclusion Body Myopathy (IBM2-MIM:600737) and Distal Myopathy with Rimmed Vacuoles (DMRV-MIM:605820) are adult onset muscle wasting disorders that are clinically and genetically indistinguishable, and often result in Quadriceps Sparing Myopathy (QSM). We refer to this phenotype collectively as IBM2/DMRV. Over the past two years, IBM2/DMRV has been associated with mutations in GNE; this gene encodes for the bifunctional enzyme GNE/MNK (EC 5.1.3.14/EC 2.7.1.60), which is the rate limiting enzyme for sialic acid biosynthesis. To date, over 30 mutations on GNE are associated with IBM2/DMRV. However, the penetrance of the mutations, and the exact relationship of the mutations to IBM2/DMRV phenotype are still ambiguous. Here we present both published and unpublished mutations, and we describe how the GNE mutations maybe related to IBM2/DMRV phenotype. We have tested 574 individuals, which include 147 patients and their immediate families. In our cohort, we identified 143 patients of Iranian heritage, and 4 patients of non-Iranian heritage. For genetic analysis, genomic samples were tested using bilateral PCR amplification of specific alleles, restriction enzyme analysis, and direct automated sequencing. Medical records were reviewed and patients interviewed. The penetrance of the GNE-M712T mutation is at most 97%. All other mutations are too rare to allow for reasonable penetrance estimation. The M712T homozygous mutation results in surprisingly diverse IBM2 phenotype with variations in sparing of specific muscle groups, age at onset of symptoms, and rate of progression. Even within a single family, the age of onset and progression can vary significantly. Based on retrospective data, pregnancy and emotional distress seem to significantly affect the rate of disease progression. Additionally, all patients with QSM screened to date have mutations on GNE, however not all GNE mutations lead to QSM. In conclusion, IBM2 phenotype is not mutation specific. It is likely that decrease in sialic acid biosynthetic flux results in increased myofiber sensitivity to degeneration. Yet unknown factors may play a significant role in IBM2/DMRV phenotype.

**A genotype-phenotype correlation for *GJB2* (connexin 26) deafness.** *K. Cryns*<sup>1</sup>, *E. Orzan*<sup>2</sup>, *A. Murgia*<sup>3</sup>, *P.L.M. Huygen*<sup>4</sup>, *F. Moreno*<sup>5</sup>, *I. del Castillo*<sup>5</sup>, *P. Chamberlin*<sup>6</sup>, *H. Azaiez*<sup>6</sup>, *S. Prasad*<sup>6</sup>, *R.A. Cucci*<sup>6</sup>, *E. Leonardi*<sup>3</sup>, *P.J. Govaerts*<sup>7</sup>, *P.H. Van de Heyning*<sup>8</sup>, *C.M. Van de Heyning*<sup>1</sup>, *R.J.H. Smith*<sup>6</sup>, *G. Van Camp*<sup>1</sup>. 1) Medical Genetics, University of Antwerp, Antwerp, Belgium; 2) Otorrhinology Unit, University Hospital of Padua, Padua, Italy; 3) Pediatrics, University of Padua, Padua, Italy; 4) Otorrhinology, University Medical Centre St Radboud, Nijmegen, The Netherlands; 5) Unidad de Genética Molecular, Hospital Ramon y Cajal, Madrid, Spain; 6) Molecular Otorrhinology Research Laboratories, Otorrhinology, University of Iowa, Iowa City, USA; 7) The Ear Group, Deurne, Belgium; 8) Otorrhinology, University of Antwerp, Antwerp, Belgium.

Mutations in *GJB2* are the most common cause of nonsyndromic autosomal recessive hearing impairment (HI), and mutation analysis of this gene is widely available as a genetic diagnostic test. HI associated with *GJB2* mutations ranges from mild to profound. To assess a possible genotype-phenotype correlation for *GJB2*, audiometric data from 277 unrelated hearing-impaired persons segregating two *GJB2* mutations were analyzed. We found that 35delG homozygotes have significantly more HI when compared to 35delG/non-35delG compound heterozygotes. Persons with two non-35delG mutations have even less HI. We observed a similar gradient of HI when we categorized mutations as inactivating (i.e. stop mutations, frame shifts) or non-inactivating (i.e. missense mutations). We could demonstrate that the *GJB2* genotype has an important impact on the degree of HI. Certain mutation combinations (including the combination of 35delG with the missense mutations L90P, V37I, or the splice-site mutation IVS1+1G>A, and the V37I/V37I genotype) are associated with significantly less HI compared to 35delG homozygous genotypes. This study is the first large and systematic analysis indicating that the *GJB2* genotype has a major impact on the degree of HI, and identifying mild genotypes. Furthermore, this study shows that it will be possible to refine this correlation and to extend it to additional genotypes. These data will be useful in evaluating habilitation options for persons with *GJB2*-related deafne.

**NOVEL MUTATIONS AND GENOTYPE:PHENOTYPE CORRELATION IN CHARCOT-MARIE-TOOTH TYPE 1B (CMT1B).** *K.T. Abe<sup>1</sup>, M.T. Magrin<sup>1</sup>, R.C.M. Pavanello<sup>1</sup>, P.E. Marchiori<sup>2</sup>, M. Zatz<sup>1</sup>*. 1) Dept. of Biology, Biosciences Institute, University of Sao Paulo, Brazil; 2) Dept.of Neurology, Faculty of Medicine, University of Sao Paulo, Brazil.

Charcot-Marie-Tooth (CMT) disease, the most frequent hereditary neuropathy, represents a group of clinically and genetically heterogeneous disorders. The most common form is CMT type 1A, caused by a duplication of 1.5Mb at 17p11.2-p12, where lies the peripheral myelin protein-22 (PMP22) gene. The second most common form, CMT1B, with an estimated prevalence of 5-10%, is caused by mutations in the myelin protein zero (MPZ) gene, at chromosome 1q21-q23. In a study of 57 patients referred to our center with a diagnosis of CMT1, we have confirmed the diagnosis of CMT1A in 58% of the cases. We are currently screening the remaining patients for mutations in the MPZ gene. The aim of this study is to estimate the frequency of CMT1B in the Brazilian population and establish genotype:phenotype correlations in affected patients. The six exons of the MPZ gene are being analyzed by SSCP and dHPLC followed by sequencing of abnormal fragments in MegaBACE automatic sequencer. Until now, we have found three novel mutations among 24 patients who were analyzed. Two are missense mutations, S54P in exon 2 and G123V in exon 3 which were not found in 340 normal chromosomes. The third one is an out-of-frame deletion of 16 pb (c.519\_535del) found in a three-year-old boy and his 39-yr-old mother. The affected mother, who has normal intelligence has a severe weakness and atrophy of lower limbs as well as reduced NCV. She can walk only short distances with support. However, her affected son presents besides difficulty in walking, a syndromic face with low ears implantation and mental retardation. These findings apparently have not been reported previously for CMT1B mutations. They give further support to the importance of genotype-phenotype correlation studies in an attempt to better understand the role of different gene domains for protein function. In addition, these preliminary findings suggest that mutations in MPZ gene (CMT1B) in Brazilian patients account for ~10% of non-CMT1A cases. Supported by: FAPESP-CEPID, PRONEX, CNPq.

**Genotype/phenotype correlations in familial tuberous sclerosis cases.** *K. Au<sup>1</sup>, C.M. Wilson<sup>1</sup>, A.T. Williams<sup>1</sup>, D.A. Johnson<sup>2</sup>, M.A. Assel<sup>1</sup>, H. Northrup<sup>1</sup>.* 1) Dept Pediatrics, The Univ Texas Medical School, Houston, TX; 2) Dept Biomathematics, The Univ Texas MD Anderson Cancer Center, Houston, TX.

Tuberous sclerosis complex (TSC) is a genetic disorder characterized by formation of benign tumors in affected organ systems. The most significant involvement resulting in morbidity and mortality occurs in the brain and kidneys. Mutations in two genes, TSC1 and TSC2, result in the TSC phenotype. Published genotype/phenotype correlations have focused on sporadic TSC cases. Further, TSC2 mutations are more frequent than TSC1 mutation(6:1)in sporadic cases. We gathered data from 32 families (123 affected individuals) with 2 affected individuals. TSC2 gene mutations were identified in 16 families (66 affecteds) and TSC1 in 11 families (41 affecteds) with 5 families (16 affecteds) having no mutation identified. Overall, our study population reported much less severe neurologic findings than studies focused on sporadic patients. We found no significant difference in TSC1 v. TSC2 mutation patients who reported seizures but we did find a statistically significant higher incidence of MR and behavioral abnormalities in TSC2 mutation patients. No TSC1 mutation patients reported renal cysts while approximately half of our TSC2 mutation patients did. Interestingly, even though the majority of findings were either the same or more severe in TSC2 mutation patients, for certain specific skin findings (ungual fibromas and shagreen patches) the TSC1 mutation patients had a more severe phenotype. Additionally, we compared mutation type between TSC2 missense mutations v. all truncating mutations (TSC2 and TSC1). TSC2 missense mutations conferred the highest risk for neurologic symptoms and TSC2 truncating mutations are associated with highest risk for disfiguring skin lesions. Further, we hypothesize that TSC2 missense mutations confer a more severe neurologic phenotype than truncating mutations possibly due to residual abnormal tuberin activity. This finding is consistent with the observation that brain tumors in TSC patients rarely exhibit loss of heterozygosity (5-10% v. 80-90% in renal tumors) suggesting that tuberin with a missense mutation may function in a dominant-negative manner.

**A novel ganglioside-induced differentiation-associated protein 1 (GDAP1) mutation associated with Charcot-Marie-Tooth disease: evidence for a founder effect in Italy.** *E. Bellone<sup>1</sup>, E. Di Maria<sup>1</sup>, R. Gulli<sup>1</sup>, P. Balestra<sup>1</sup>, D. Cassandrini<sup>1</sup>, M. Bado<sup>2</sup>, L. Doria-Lamba<sup>3</sup>, A. Schenone<sup>1</sup>, F. Ajmar<sup>1</sup>, P. Mandich<sup>1</sup>.* 1) Dept Neurosci Ophthalmol Genet, Univ Genova, Genova, Italy; 2) Unit for Neuromusc Diseases, Inst G. Gaslini, Italy; 3) Divisione e Cattedra di Neuropsichiatria Infantile, Inst G. Gaslini, Univ Genova, Italy.

Mutations in a gene encoding a novel protein of unknown function, the ganglioside-induced differentiation-associated protein 1 gene (GDAP1), are associated with one of the autosomal recessive forms of Charcot-Marie-Tooth disease (CMT4A). The function of GDAP1 in the peripheral nervous system is still unknown. A series of 76 Italian patients with severe early onset polyneuropathy and possible autosomal recessive inheritance was screened for mutations in the GDAP1 gene. All patients were negative for the 17p11.2 duplication and for mutations in the MPZ, GJB1, PMP22 and EGR2 genes. The entire coding region, including exon-intron boundaries, was examined by single strand conformation polymorphism (SSCP) and direct sequencing. An altered SSCP pattern and subsequent direct sequencing detected, in three unrelated patients, a T>G transversion (c.347 TG) which causes an aminoacidic change from methionine to arginine at codon 116 (M116R). The M116R mutation showed cosegregation with the disease in all three pedigrees and was not present in 314 control chromosomes. All patients showed early onset of the disease with pronounced foot deformities and impaired deambulation. The neurophysiological study demonstrated primary axonal damage and primary or secondary demyelination. The sural nerve biopsies revealed different abnormalities ranging from axonal de- and regeneration to demyelination and remyelination, the most prominent feature being a severe loss of larger fibers. Detailed clinical, electrophysiological and neuropathological features of the three patients will be presented. Haplotype analysis of the GDAP1 locus with three short tandem repeat markers and an intragenic single nucleotide polymorphism showed a common disease haplotype, suggesting that these families, coming from the same Italian region (Campania), may have a common ancestor.

**Molecular analysis of 4q35 rearrangements: sensitivity and specificity of EcoRI/BlnI test for FSHD. G.**

*Galluzzi<sup>1,2</sup>, M. Rossi<sup>1</sup>, L. Colantoni<sup>1,2</sup>, B. Merico<sup>1</sup>, P. Tonali<sup>1</sup>, L. Felicetti<sup>1,2</sup>, E. Ricci<sup>1,2</sup>.* 1) Department of Neurosciences, Catholic University, Rome, Italy; 2) Center for Neuromuscular Diseases, UILDM-Rome, Italy.

FSHD is one of the most common forms of muscular dystrophy, with a prevalence of about 1:20,000. While FSHD still remains a gene-orphan disease, considerable progress has been made in developing a reliable molecular test since a DNA rearrangement at 4q35 locus has been unequivocally associated with the disease. The test consists in the detection, after hybridization with probe p13E-11, of a shortened EcoRI, BlnI-resistant fragment (10 to 40 kb in FSHD patients, > than 40 kb in normal individuals). While the sensitivity of this molecular approach is very high (over 96%), its specificity has never been adequately considered. In our work we studied 151 families by PFGE. In 120 families (group A) a diagnosis of FSHD had been performed on the base of clinical features, according to ENMC Consortium criteria. The remaining 31 (group B) did not fulfill the diagnostic criteria for FSHD. 210 affected and 142 unaffected individuals from the 120 FSHD families belonging to group A and 87 individuals from the 31 belonging to group B were analyzed. In addition, we studied 71 unrelated normal individuals. Group A: an EcoRI BlnI-resistant fragment, 13 to 41 kb in size, was detected in 115 out of 120 families. Unexpectedly, in 40 individuals belonging to 17 families we found 23 BlnI-resistant, EcoRI fragments sized 23 to 41 kb in addition to those causally associated with the disease. It is worth noting that 21 out of 40 individuals carried exclusively a 23-41 kb fragment different from that associated with the disease and none was affected by FSHD. Group B: eight EcoRI, BlnI-resistant fragments in the range 22 to 41 kb were found in individuals belonging to 7 of the 31 families examined. An EcoRI,BlnI-resistant fragment sized 30 to 41 kb was detected in 5 out of 71 normal individuals. Due to the existence of overlap of 4q35 EcoRI fragments between patients and normal individuals, a diagnosis of FSHD should not be based on molecular grounds alone in families not fulfilling the diagnostic criteria for FSHD.

**Molecular characterization of p63 mutations found in six different human disorders involving limb, ectodermal, and lip/palate abnormalities.** *P.H.G. Duijf<sup>1</sup>, V. Doetsch<sup>2</sup>, J.A. McGrath<sup>3</sup>, T. Rinne<sup>1,4</sup>, J. Celli<sup>1</sup>, A. Yang<sup>5</sup>, F. McKeon<sup>5</sup>, E. van Beusekom<sup>1</sup>, J. Schalkwijk<sup>6</sup>, P. Propping<sup>7</sup>, F. Gurrieri<sup>8</sup>, B.C.J. Hamel<sup>1</sup>, H.G. Brunner<sup>1</sup>, H. van Bokhoven<sup>1</sup>.* 1) Dept Human Genetics 417, University Medical Centre Nijmegen, Nijmegen, The Netherlands; 2) Pharmaceutical Chemistry, San Francisco, CA, USA; 3) Dermatology, London, UK; 4) Medical Genetics, Turku, Finland; 5) Cell Biology, Boston, MA, USA; 6) Dermatology, Nijmegen, The Netherlands; 7) Human Genetics, Bonn, Germany; 8) Medical Genetics, Rome, Italy.

The p63 gene is a homologue of the p53 tumor suppressor gene. In contrast to p53, p63 plays an important role in development, particularly in the development of the limbs, skin, sweat and sebaceous glands, hair, and lip/palate. We have previously shown that Ectrodactyly, Ectodermal dysplasia, Cleft lip/palate (EEC) syndrome is caused by mutations in the p63 gene. Subsequently, p63 mutations were identified in five related disorders, including Hay-Wells/AEC syndrome, ADULT syndrome, Limb-Mammary Syndrome (LMS), Rapp-Hodgkin Syndrome (RHS), and Split Hand/Foot Malformation (SHFM). Clear genotype-phenotype correlations can be recognized for these mutations. EEC syndrome is caused by missense mutations in the DNA binding domain resulting in a loss of DNA binding activity, while ADULT syndrome is caused by a gain-of-function mutation in the DNA binding domain. In addition, AEC syndrome and RHS are predominantly caused by missense mutations in the SAM protein-protein interaction domain of p63. Also, most of the SHFM and LMS mutations are unique for these disorders. Moreover, the considerable clinical overlap between LMS and ADULT syndrome is paralleled by the comparable positions of the mutations in these two syndromes. Currently, we are investigating the functional consequences of p63 mutations in more detail. We have performed a yeast-two-hybrid screen to identify proteins that interact with the C-terminus of the p63 splice variant. Additionally, functional assays are being performed to investigate the relationship between LMS mutations and the gain-of-function mutation that causes ADULT syndrome. These studies will gain more insight into the normal and disrupted pathways involving p63.

**Quantitative analysis of allelic unbalance by Primer Extension and DHPLC suggests a high incidence of somatic mosaicism in tuberous sclerosis.** A. Allavena<sup>1</sup>, S. Lanzardo<sup>1</sup>, S. Padovan<sup>1,2</sup>, M. Barberis<sup>1</sup>, L. Longa<sup>1</sup>, C. Michielotto<sup>1</sup>, A. Brusco<sup>1</sup>, B. Ferrando<sup>1</sup>, N. Migone<sup>1</sup>. 1) Dept Genetics, Biol & Biochem, Univ Torino, Torino, Italy; 2) CNR, Torino.

The footprint of somatic mosaicism is the unbalance of copy number between the wild type and mutated alleles. In order to assess the incidence of somatic mosaicism in tuberous sclerosis we compared the sensitivity of two methods: DHPLC, run at 2 temperatures, and Primer Extension (PE), using forward and reverse primers adjacent to the mutation. Three pairs of wild type and mutated alleles from mosaic parents of TSC probands, representative of 2 types of DHPLC profiles, difficult and easy to read - showing either a distorted single pick, or 2 to 4, well separated picks, respectively - were cloned and mixed at decreasing ratios of mut : wt alleles. Each mix was tested by DHPLC and PE, and the results compared with those obtained from peripheral blood DNA of the mosaic parents, the probands and controls homozygous for the wt allele. The window sensitivity of PE appeared consistently wider (90% to 10% of the mut cell fraction) than that of DHPLC, whose range of detectable mosaicism in presence of difficult profiles was reduced to 40% - 20% of the mut cells. These data suggest that, a) mut-pos or mut-neg cell fractions 10% are easily missed by standard DHPLC analysis, b) the ascertainment of allelic unbalance largely depends on the availability of a balanced heterozygote for the same mutation to be used as heteroduplex profile of reference. Since most TSC mutations are unique, mosaics have a higher chance to be recognized in multiplex families than in a sporadic patient. Indeed, we found a higher prevalence of somatic mosaicism in multiplex TSC2 families (9/36; 25%) than among de novo TSC2 probands (10/153; 6,5%). It is reasonable to assume that sporadic mosaics tend to have minor signs, so that most of them are identified after the birth of a baby with a classical phenotype. In conclusion, somatic mosaicism in TSC is likely underestimated and the multiplex family data suggest that 1 of 4 TSC2 patients might originate through a post-zygotic mutation.

***HAMP* candidate as modifier gene in adult-onset form of hereditary hemochromatosis.** S. Jacolot, G. Le Gac, I. Quéré, C. Mura, C. Férec. INSERM 0115, Université de Bretagne Occidentale, Etablissement Français du Sang, Centre Hospitalier Universitaire, Brest, France.

Background: Hereditary Hemochromatosis is a genetically heterogeneous disease of iron metabolism. The most common form of the disorder, called hereditary hemochromatosis type I, is an adult-onset form which has mainly been associated with the C282Y *HFE* gene mutation. The non-*HFE*-related forms include a juvenile-onset form associated with mutations in the *HAMP* gene. The goal of this study was to evaluate the role of the *HAMP* gene in adult-onset forms of primary iron overload. Methods: We studied the *HAMP* gene in 392 C282Y homozygous patients and in 300 control subjects. We designed a D-HPLC assay to scan the *HAMP* coding sequence and sequenced DNA displaying an altered profile. Results: We found 5 patients out of 392 carrying one mutation in the *HAMP* gene. Among the three *HAMP* gene mutations, two were novel (R59G and G71D) and one was previously described (R56X); they were not detected in the control population. Discussion: Our result revealed that, the *HAMP* gene might act as a modifier gene in phenotypes related to Hereditary Hemochromatosis type I. We further highlighted that, based on the digenic model of inheritance already described in other human genetic disorders, the association of mutations in the *HFE* and *HAMP* genes may lead to an adult-onset form of primary iron overload.

**Evidence of a dominant negative effect of delta lamin A reflects the pathogenic mechanism underlying Hutchinson-Gilford progeria syndrome (HGPS).** *W. Ju, W.T. Brown, N. Zhong.* Department of Human Genetics, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY10314.

HGPS is a very rare premature aging syndrome with autosomal dominant inheritance. Recently, HGPS has been found to result from genetic defects of the LMNA gene, which encodes nuclear envelope proteins lamin A/C. A silent mutation, c2063C>T that results in G608G, generates a cryptic splicing site in exon 11 of LMNA, and consequently truncates 50 amino acids near the C-terminus of lamin A, was identified in ~80% of HGPS alleles. We have undertaken a study to demonstrate the dominant negative effect of this mutation. We transfected either the mutant LMNA construct that expresses a delta lamin A derived from the mutation c2063C>T, or a normal LMNA construct into a normal human fibroblast culture. Immunostaining was performed to study the architecture of nuclear envelopes. Bleb formations of the nuclear envelope, identical to that seen in cells derived from HGPS patients, were shown in the normal cells transfected by mutant LMNA but not in those transfected by normal LMNA. After 48-72 hours of transfection, co-immunoprecipitation studies were carried out with transfected cell extracts and antibodies to the various lamina proteins: lamin A/C, lamin B1, SREBP1, LAP2a, and Emerin, and to control proteins LAMP1 and BI1. No differences were identified, perhaps reflecting the abundance of the normal allele in HGPS cells. The finding of abnormal nuclear architecture following transfection of the mutant allele provides evidence that the mutant LMNA has a dominant negative effect in HGPS.

**Locus and allelic influences on clinical severity of Autosomal Dominant Polycystic Kidney Disease (ADPKD).** *N. Lamb*<sup>1</sup>, *W. Paxton*<sup>2</sup>, *D. Rizk*<sup>2</sup>, *J. Du*<sup>2</sup>, *J. Ladson*<sup>2</sup>, *S. Langley*<sup>2</sup>, *A. Amin*<sup>1</sup>, *E. Boerwinkle*<sup>3</sup>, *A. Chapman*<sup>2</sup>. 1) Human Genetics, Emory Univ, Atlanta, GA; 2) Medicine, Emory Univ, Atlanta, GA; 3) University of Texas Health Science Center, Houston, TX.

ADPKD is characterized by renal cyst formation and growth that leads to massive renal enlargement and end stage renal disease (ESRD). Renal volume (RV) is a significant marker for disease progression in ADPKD. ADPKD is genetically heterogeneous, with mutations in PKD1 and PKD2 occurring in 85% and 15% respectively, of cases. Mutations in PKD2 are believed to result in milder disease with regard to age of ESRD. Recent studies indicate mutation type but not position influences age of ESRD outcome in PKD2 individuals. However, time to ESRD is a relatively late clinical event in ADPKD. Therefore, we examined locus and allelic heterogeneity using renal volume, measured by magnetic resonance imaging as an early predictor of disease severity. 272 ADPKD individuals from 185 families were clinically evaluated, including measurements for RV and renal function. The PKD2 promoter, coding regions, and intron/exon boundaries were sequenced for all individuals. Thirty-one individuals, from 20 families (11%) were identified with mutations in PKD2, consistent with previous gene linkage analysis. Remaining individuals were assumed to have PKD1 mutations. PKD1 and PKD2 patients demonstrated significant and similar volume-function interaction (PKD1:  $r = -0.68$ ,  $p < 0.0001$ ; PKD2:  $r = -0.82$ ,  $p < 0.0001$ ), indicating a similar structure-function relationship. We examined the effect of mutation type and location by comparing RV in PKD2 individuals according to missense, truncating, or splice-site mutations. Significantly smaller median RV was found in individuals with splice site mutations (649mls) compared to missense (1694mls) and truncating (1111mls), suggesting that mutations that alter splicing are associated with milder disease. Missense and truncating mutations located nearer the 3' end of PKD2 also demonstrated higher RV, suggestive of a more severe outcome ( $r = 0.16$ ,  $p < 0.004$ ). Taken as a whole, these data suggests mutation type and location can provide useful prognostic indicators for individuals affected with PKD2.

**Molecular characteristics of the *OPA1* gene in the Korean patients with autosomal dominant optic atrophy.** *M.W. Seong*<sup>1</sup>, *J.Y. Kim*<sup>1,3</sup>, *J.M. Hwang*<sup>2</sup>, *H.S. Ko*<sup>1</sup>, *S.S. Park*<sup>1</sup>. 1) Laboratory Medicine, Seoul National University Hospital Clinical Research Institute, Seoul, Korea; 2) Ophthalmology, Seoul National University Bundang Hospital, Sungnam, Korea; 3) Blood Transfusion Research Institute, Korean Red Cross, Seoul, Korea.

Autosomal dominant optic atrophy (ADOA) is the commonest form of inherited optic neuropathy that features low visual acuity leading in many cases to legal blindness. A major locus mapped to chromosome 3q28 and the gene responsible, *OPA1* gene (MIM 165000), was recently identified. To identify the molecular characteristics of the *OPA1* gene, we investigate spectrum and genotype-phenotype correlation in Korean patients with ADOA. A total of 16 ADOA families were analyzed. Nine of them were from pedigrees with affected in at least two subsequent generations, two were from families with an affected sibling and four patients were sporadic. Family history was unavailable in one case. We also analyzed 14 patients with Leber's hereditary optic neuropathy (LHON) who had not harbor the primary LHON mitochondrial DNA mutations. All 28 coding exons and the flanking regions of the *OPA1* gene were amplified. Mutations were screened by SSCP and identified by direct sequencing. We identified eight different mutations among 16 patients: three splicing, three missense, one nonsense, and one frameshift mutation. All of them were novel except one (87.5%). A majority of these mutations were truncative (50%) and located throughout the gene (exons 2-25). Two of three missense mutations were found in the GTPase domain of the *OPA1* protein (exon 8-12). None of them was detected 100 normal control chromosomes. There is no remarkable genotype-phenotype correlation. Eighteen polymorphisms were found, nine of which were novel. No mutations were found in any LHON patient. *OPA1* mutations and polymorphisms in Koreans reveal a different spectrum from that in Caucasians reported and do not show a founder effect, suggesting a random mutational event occurring recently. A majority of these are truncative mutations and support that the haploinsufficiency may represent a major pathomechanism for ADOA.

**B-CAM/LU modifies Sickle Cell Disease severity.** *L.M. De Castro<sup>1</sup>, J. Jonassaint<sup>1</sup>, S. Rainey<sup>3</sup>, R. Motley<sup>2</sup>, S. Jones<sup>2</sup>, J. Galloway<sup>1</sup>, L. Zhang<sup>1</sup>, T. Uchiyama<sup>1</sup>, J.R. Eckman<sup>3</sup>, E.P. Orringer<sup>2</sup>, A.E. Ashley-Koch<sup>1</sup>, J.M. Vance<sup>1</sup>, M.J. Telen<sup>1</sup>.* 1) Duke University Medical Center, Durham, NC; 2) University of North Carolina, Chapel Hill, NC; 3) Emory University, Atlanta, GA.

B-CAM/LU mediates sickle red blood cell (RBC) adhesion to subendothelial laminin and is a candidate for modifying sickle cell disease (SCD) severity. We examined two polymorphisms in B-CAM/LU, the Lutheran blood group antigen ( $\text{Lu}^a / \text{Lu}^b$ ) and the Auberger polymorphism ( $\text{Au}^a / \text{Au}^b$ ), in 179 patients with Hb SS or Hb S<sup>0</sup>-thalassemia for associations with cerebrovascular events, acute chest syndrome, hand-foot syndrome, priapism, leg ulcers, and aseptic necrosis. For  $\text{Lu}^a / \text{Lu}^b$ , there is evidence for association with cerebrovascular events (stroke, transient ischemic attack or seizure, primarily due to an association with stroke ( $p=0.01$  genotype)) and with priapism ( $p=0.01$ ). However, the association with priapism was due to the opposite allele from stroke ( $\text{Lu}^a$  vs  $\text{Lu}^b$ , respectively). For  $\text{Au}^a / \text{Au}^b$ , preliminary results also suggested an association with priapism ( $p=0.004$  genotype). To further examine these data, we measured RBC adhesion to immobilized laminin (in a flow chamber assay) in steady state samples of 23 patients at a shear stress of 1 dyne/cm<sup>2</sup>. The mean laminin binding of  $\text{Lu}^b / \text{Lu}^b$  patients (385.6 cells/cm<sup>2</sup>) was not significantly different from  $\text{Lu}^a / \text{Lu}^b$  patients (467.3 cells/cm<sup>2</sup>). No  $\text{Lu}^a / \text{Lu}^a$  patients were observed. Examination of mean laminin binding as a function of Au genotype was also not statistically significant but there was a trend of increased mean laminin binding with increasing number of  $\text{Au}^a$  alleles ( $\text{Au}^b / \text{Au}^b$ : 285 cells/cm<sup>2</sup>;  $\text{Au}^a / \text{Au}^b$ : 437.4 cells/cm<sup>2</sup>;  $\text{Au}^a / \text{Au}^a$ : 523 cells/cm<sup>2</sup>). These data suggest that the  $\text{Lu}^a / \text{Lu}^b$  and  $\text{Au}^a / \text{Au}^b$  polymorphisms in B-CAM/LU may be important modifiers of the clinical outcomes of patients with SCD and that different clinical sequelae may have different underlying etiologic mechanisms. Further, although not statistically supported by the functional data in the small sample examined here, the genotypic associations that we have observed are most likely due to variations in RBC adhesion to laminin.

**Examination of VCAM1 as a modulator of stroke risk in sickle cell disease.** *M.J. Telen<sup>1</sup>, A.E. Ashley-Koch<sup>1</sup>, L. Zhang<sup>1</sup>, J. Jonassaint<sup>1</sup>, S. Rainey<sup>3</sup>, R. Motley<sup>2</sup>, S. Jones<sup>2</sup>, J. Galloway<sup>1</sup>, T. Uchiyama<sup>1</sup>, J.R. Eckman<sup>3</sup>, E.P. Orringer<sup>2</sup>, J.M. Vance<sup>1</sup>.* 1) Duke University Medical Center, Durham, NC; 2) University of North Carolina, Chapel Hill, NC; 3) Emory University, Atlanta, GA.

Sickle cell disease (SCD) results from a single gene mutation, yet there is remarkable variation in the clinical course of patients with this disease. Stroke occurs in only 10-20% of patients but is a significant cause of morbidity and mortality starting in childhood. Identifying molecular predictors of such clinical outcomes will have important implications for the treatment of patients with SCD. Taylor et al (2002) previously reported an association between stroke and a non-synonymous SNP (G1238C) in the VCAM1 cell adhesion molecule in 102 patients with Hb SS. We have examined this SNP in our own dataset of 179 patients with Hb SS or Hb S $\beta$ 0-thalassemia. Uncorrected chi-square or fisher-exact p-values are reported as appropriate. Preliminary analysis did not detect significant association between G1238C and the outcome of stroke alone (p=0.06 genotype). However, when broadening the analysis to include any type of cerebrovascular event (CVA; defined as stroke, transient ischemic attack or seizure), there was evidence for an association at the genotype level (p=0.02). Interestingly, we also observed evidence for association with occurrence of hand-foot syndrome (p=0.007 genotype). However, the C allele was associated with CVA in our dataset and that of Taylor et al (2002), but the G allele was associated with hand-foot syndrome. We did not detect associations with G1238C and aseptic necrosis of the hip or shoulder, acute chest syndrome, leg ulcers, or priapism. These results suggest that differing biological mechanisms may underlie the etiology of these sickle complications. Thus, we conclude that VCAM1, and potentially other cell adhesion molecules, are likely to play an important role in some but maybe not all complications of SCD. Further examination in larger datasets will be needed to confirm this hypothesis.

**Identifying dosage-sensitive genes in the Smith-Magenis Syndrome region.** *P. Fonseca, K. Walz, J. Lupski.* Dept Human Molecular Genetics, Baylor Col Medicine, Houston, TX.

The Smith-Magenis Syndrome (SMS) is thought to be a contiguous gene syndrome associated with a 4Mb deletion in chromosome 17p11.2. Haploinsufficiency of one or more genes in the region seems to be responsible for the etiology of the disease. The 1Mb critical region is syntenic to a region of murine chromosome 11 and the number and order of the genes are highly conserved. A deletion and its reciprocal duplication were engineered in the mouse spanning the genomic interval commonly deleted in SMS patients to model the disease and to study dosage effects of the genes in the region. Heterozygous deleted (Df(11)/+) mice present craniofacial abnormalities, seizures, obesity and a shorter period length when compared to wild-type littermates. Duplicated (Dp(11)/+) animals are underweight and show hyperactivity and learning disabilities. Analysis of Df(11)/Dp(11) animals suggests that most of the observed phenotypic features result from gene dosage effects. The three strongest candidates for dosage effects genes are Rai1 (Retinoic Acid Induced), Drg2 (Developmentally Regulated G protein) and Rasd1 (Dexamethasone-induced RAS-related protein). The RASD1 protein is hormonally regulated and is involved in the Erk-MAP kinase pathway. DRG2 is a GTP-binding protein with a potential role in early neurogenesis. Rai1 is retinoic acid induced and is specifically expressed in the mouse brain during development. Retinoic acid is known to be involved in the development of craniofacial abnormalities. Recently, dominant frameshift mutations leading to protein truncation in the RAI1 gene were identified in patients with many features observed in SMS. We used BACs containing the three candidate genes to generate overexpressing mice and to perform in vivo complementation, by crossing transgenic animals with the engineered heterozygous deleted mice. The F1 generation is being analyzed for the phenotypic features found in Df(11)/+ and Dp(11)/+ mice. The use of transgenic technology will provide a powerful means to assess gene dosage effects in the mouse, as well as identifying the gene responsible for the phenotype in the SMS mouse model through complementation analysis.

**A CLUSTER OF MUTATIONS IN THE UMOD GENE CAUSES FAMILIAL JUVENILE HYPERURICEMIC NEPHROPATHY WITH ABNORMAL EXPRESSION OF UROMODULIN.** *K. Dahan<sup>1</sup>, O. Devuyst<sup>2</sup>, M. Smaers<sup>1</sup>, G. Loute<sup>3</sup>, J.M. Poux<sup>4</sup>, M.F. Gagnadoux<sup>5</sup>, D. Chauveau<sup>6</sup>, J.P. Cosyns<sup>7</sup>, C. Antignac<sup>8</sup>, Ch. Verellen-Dumoulin<sup>1</sup>, Y. Pirson<sup>2</sup>.* 1) Dept Genetics, UCL Saint-Luc Hosp, Brussels, Belgium; 2) Division of Nephrology, UCL Saint-Luc Hosp, Brussels, Belgium; 3) Dept of Nephrology, Princesse Paola Hospital, Aye, Belgium; 4) Dept of Nephrology, Frejus, France; 5) Dept of Pediatric Nephrology, Hopital Necker, Paris, France; 6) Division of Nephrology and INSERM U507, Hopital Necker, Paris, France; 7) Dept of Pathology, UCL Saint-Luc Hospital, Brussels, Belgium; 8) Universite Paris V, Necker Hospital, INSERM U574 and Department of Genetics, Paris, France.

Familial juvenile hyperuricemic nephropathy (FJHN [MIM 162000]) is an autosomal-dominant disorder characterized by abnormal tubular handling of urate and late development of chronic interstitial nephritis leading to progressive renal failure. We and others previously identified a locus for FJHN on chromosome 16p12 close to the *MCKD2* locus, which is responsible for a variety of autosomal dominant medullary cystic kidney disease (MCKD2). *UMOD*, the gene encoding the Tamm-Horsfall/uromodulin protein maps within the FJHN/MCKD2 critical region. Mutations in *UMOD* were recently reported in 4 families with FJHN/MCKD2 disease. We have identified 8 novel mutations in *UMOD* (7 missense and one in-frame deletion) among FJHN families, clustering in the highly conserved exon 4. The consequences of *UMOD* mutations on uromodulin expression were investigated by immunohistochemistry in 3 renal biopsies from 2 families. There was a markedly increased expression of uromodulin in a cluster of tubule profiles, suggesting an accumulation of the protein in tubular cells. Consistent with this observation, urinary excretion of uromodulin was significantly decreased. In conclusion, our study points out to a mutation clustering in the exon 4 of *UMOD* as a major genetic defect in FJHN. Identification of *UMOD* mutations thus allows an accurate diagnosis and genetic counselling in a significant subset of FJHN/MCKD2 disease. These data give new insights on the function of uromodulin and the renal handling of urate.

**Neonatal Onset Multisystem Inflammatory Disease (NOMID): further evidence for genetic heterogeneity and search for novel additional NOMID causing genes.** *I. Aksentijevich<sup>1</sup>, F. Austin<sup>1</sup>, J.J. Chae<sup>1</sup>, R. Goldbach Mansky<sup>2</sup>, D.L. Kastner<sup>1</sup>.* 1) Genetics & Genomics Branch, NIH/NIAMS, Bethesda, MD; 2) Office of the Clinical Director NIH/NIAMS, Bethesda, MD.

Neonatal Onset Multisystem Inflammatory Disease (NOMID/CINCA syndrome) is a rare autoinflammatory disease characterized by fever, meningitis, uveitis, sensorineural hearing loss, urticarial rash, and a characteristic deforming arthropathy. NOMID is caused by missense mutations in *CIAS1* (*NALP3*, *PYPAF1*), which encodes cryopyrin. NOMID is allelic with Muckle-Wells (MWS) and familial cold autoinflammatory syndrome (FCAS). More than 30 mutations causing MWS/FCAS/NOMID have been reported to date, all in the NACHT domain of the protein. We previously reported 6 mutations in 13 NOMID cases, 5 of which were novel. Consistent with the recently discovered role of *CIAS1* in IL-1 regulation, we found evidence for increased IL-1, as well as TNF, IL-3, IL-5, and IL-6, in monocytes from a patient with the D303N mutation. Subsequently, we have analyzed 8 more NOMID patients and found mutations in only 4. Two of the mutations are novel (F523C and G326E). Monocytes from these patients showed increased levels of IL-1 precursor by Western blot analysis regardless of whether a *CIAS1* mutation was present. Overall, only 50% (10/21) of our NOMID patients are *CIAS1* mutation positive, which further strengthens evidence for genetic heterogeneity. Cryopyrin is a member of the recently identified family of genes known as PYPAFs or NALPs, with more than 14 members identified so far. We screened our *CIAS1* mutation negative patients for mutations in the NACHT domains of several of the NALP molecules that are highly homologous to cryopyrin or that have similar tissue expression profiles as possible NOMID candidate genes, including NALP1/DEFKAP, NALP2/PYPAF2, NALP4/PAYPAF4, NALP6/PYPAF5, NALP12/PAYPAF7, as well as apoptosis-associated speck-like protein with a CARD (ASC), which interacts with cryopyrin to regulate apoptosis and proinflammatory caspases. We have identified several missense substitutions in these molecules, and we are currently screening controls and evaluating the functional consequences of these variants.

**Genetic Testing for Hereditary Hyperferritinemia Cataract Syndrome: a Common Mutation and Coincident Hemochromatosis Genotype.** *D.G. Brooks*<sup>1</sup>, *D. Stambolian*<sup>2</sup>. 1) Medical Genetics; 2) Ophthalmology & Genetics, Univ Pennsylvania, Philadelphia, PA.

Hereditary Hyperferritinemia Cataract Syndrome (HHCS) is defined by dominant inheritance of cataracts, elevated serum ferritin and specific mutations of the L-ferritin gene, HFERL. Mutation of the iron responsive element in the 5' UTR of the HFERL mRNA causes disinhibition of L-ferritin translation. Serum ferritin reaches up to 2,300 mg/dL in HHCS, often resulting in misdiagnosis as hemochromatosis. Four observations have been made in the largest series of patients undergoing genetic testing for HHCS in the U.S.: Of 11 referrals accepted with the combination of elevated ferritin and cataract 9 had mutations in HFERL. A novel variant, C33T, has subsequently been confirmed in another HHCS family. Four of 9 kindreds carry the G32A mutation and all of these families have German and/or English ancestry; G32A is also the most common HFERL mutation in French and Italian HHCS families. Of note, 3 of the 7 G32A-linked HHCS families are also segregating the 2 common hemochromatosis (HFE) mutations. Each family includes at least one HHCS patient with the compound heterozygous HFE genotype C282Y/H63D. Two of these individuals have the highest ferritin levels ever reported in HHCS (2,750-4,700). Phlebotomy treatment of possible iron overload achieved only 10-25 percent reductions of ferritin in 2 such patients indicating mild iron loading. Requiring that cases have both cataract and hyperferritinemia produces high sensitivity for HHCS mutation testing. Haplotype analysis is planned to differentiate a founder effect of the common G32A variant from recurring mutation. We have identified 3 individuals with genotypes predisposing to 2 genetic diseases that both increase ferritin: HHCS and hemochromatosis. Such individuals are at very high risk of being misdiagnosed as hemochromatosis and inappropriately treated by phlebotomy therapy. While this case series is too small to draw conclusions, it is possible that a selective advantage is conferred by high ferritin levels in HHCS that may buffer high free iron concentrations in individuals with concurrent hemochromatosis mutation(s).

**A genome wide scan for Familial Idiopathic basal Ganglia Calcification (Fahr's disease) identifies new candidate regions and confirms genetic heterogeneity.** *J.R.M. Oliveira<sup>1</sup>, S. Hopfer<sup>1</sup>, J. Papp<sup>2</sup>, E. Spiteri<sup>1</sup>, J. Klepper<sup>3</sup>, J.*

*Gilbert<sup>4</sup>, Z.K. Wszolek<sup>4</sup>, M. Hutton<sup>4</sup>, F. Boller<sup>4</sup>, M.J. Sobrido<sup>1</sup>, D.H. Geschwind<sup>1</sup>.* 1) David Geffen Medical School Neurology Dept, University of California, Los Angeles, CA; 2) Department of Human Genetics, UCLA, Los Angeles, CA; 3) Department of Pediatric Neurology, University of Essen, Essen, Germany; 4) Department of Neurology, Mayo Clinic, Jacksonville, FL.

Fahr's disease, or Idiopathic Basal Ganglia Calcification (IBGC), is a neurological condition characterized by calcifications in the basal ganglia nucleus, cerebellum and occasionally the cerebral subcortical white matter. Clinical manifestations include dystonia, parkinsonism, and neurobehavioral symptoms such as psychosis. The first locus associated with IBGC resides on the long arm of the chromosome 14 (IBGC1 - Geschwind et al 1999). Considering a family of Spanish origin, with probable linkage to the same region, the critical region may be narrowed to 10.9 cM (Sobrido et al 2003). **PURPOSE:** To assess genetic heterogeneity in IBGC and to define new candidate regions we performed linkage analysis in several families with IBGC. **METHODS:** A 10cM genome wide scan was performed in 4 families with a total of 47 subjects using a ABI MD 10 marker panel. An additional family with 14 more subjects is still under analysis. Using LINKAGE and SIMWALK2 we performed multipoint and two point analysis. **RESULTS:** The largest single-point lod score was observed on chromosome 7 with a LOD score of 3.023 at marker D7S519 in the FB2 family (10 affected). The minimal interval defined by haplotype analysis is between the markers D7S484 and D7S506, spanning about 20cM. Three smaller families, with a total of 16 affected, had a maximum multipoint LOD score of 3.487 on chromosome 9, at a position roughly midway between D9S157 and D9S171. Haplotype analysis shows that these families share a common region around 16 cM between the markers D9S171 and D9S1817. These results reinforce preliminary studies demonstrating genetic heterogeneity in Fahr's disease and report two potential new candidate regions, one of them shared by three families.

**DNA sequence variants in AGTR2 are not associated with X-linked mental retardation.** *S. Huang*<sup>1</sup>, *M. Peng*<sup>2</sup>, *M.X. Zoleikhaeian*<sup>1</sup>, *W. Sun*<sup>1</sup>, *C.M. Strom*<sup>1</sup>. 1) Dept Molecular Genetics, Quest Diagnostics, San Juan Capistrano, CA; 2) Harvard Partners Center for Genetics and Genomics, Harvard Medical School, Cambridge, MA.

Angiotensin II is a potent regulator for many of the well-known stimulatory physiological activities including secretion of aldosterone, vasoconstriction, and renal sodium reabsorption. These actions are primarily mediated by Angiotensin Receptor I (AGTR1), the receptor function of a second receptor AGTR2 was largely unknown. Recent studies have suggested that one frame shift and three missense mutations of AGTR2 may be associated with X-linked mental retardation (Vervoort et al., 2002). To assess the effect of AGTR2 on brain development and cognitive function, we screened DNA from a cohort of 500 male patients submitted for Fragile X testing that were negative for FMR1 expansion. As controls, we also screened DNA from presumably nonretarded 500 males and normal females. Single nucleotide primer extension methodology was utilized to screen the previously described sequence variants in the AGTR2 gene (Vervoort et al., 2002). We detected hemizygotes for the frame shift mutation, 402delT, and hemizygotes of the missense mutation, G62T, in male patients both with or without mental retardation and in similar frequencies. DNA from 1000 normal female controls showed the G62T mutation in about 10%. Nishimura et al. (1999) also showed that in human a large fraction of the general population (42%) carry another functionally significant mutation (A-1332G) in AGTR2 gene. Collectively these results argued that mutations in AGTR2 gene are not associated with X-link mental retardation. The possible role of AGTR2 in the central nervous system still need to be clarified.

**Mutations in the human *LARGE* gene cause a form of congenital muscular dystrophy with severe mental retardation and abnormal glycosylation of -dystroglycan (MDC1D).** C.A. Longman<sup>1</sup>, M. Brockington<sup>1</sup>, C. Kennedy<sup>2</sup>, C. Jimenez<sup>1</sup>, S. Torelli<sup>1</sup>, C. Sewry<sup>1</sup>, S. Brown<sup>1</sup>, F. Muntoni<sup>1</sup>. 1) Dubowitz Neuromuscular Centre, Imperial College, London, UK; 2) Pediatric Neurology Department, Southampton, UK.

The congenital muscular dystrophies (CMD) are a heterogeneous group of autosomal recessive disorders, four of which are due to defects in genes encoding known or putative glycosyltransferases. Fukuyama CMD, Muscle-Eye-Brain disease and Walker-Warburg syndrome, severe forms with eye abnormalities and neuronal migration defect, are due to mutations in *fukutin*, *POMGnT1* and *POMT1* respectively. Mutations in the fukutin-related protein (*FKRP*) gene cause congenital muscular dystrophy 1C and limb girdle muscular dystrophy 2I, forms not typically associated with brain involvement. The expression of -dystroglycan is altered in these disorders and evidence indicates this is due to its abnormal glycosylation.

The Large<sup>myd</sup> mouse has a homozygous deletion in the gene encoding a putative glycosyltransferase, Large. Large<sup>myd</sup> mice have a progressive myopathy, neuronal migration defect and abnormal glycosylation of -dystroglycan. Thus, the human *LARGE* gene was a strong candidate for involvement in human muscular dystrophies with abnormal glycosylation of -dystroglycan.

We have identified *LARGE* mutations in a patient with congenital muscular dystrophy. She is a 17 year old girl of non-consanguineous British parents with profound mental retardation and extensive white matter changes on brain MRI but apparently normal brain structure. She is a compound heterozygote for a non-conservative missense mutation, G1525A (Glu>Lys), and G2000insT (which causes a frameshift and premature termination), both within the putative second catalytic domain of Large. Immunolabelling of glycosylated -dystroglycan is reduced in skeletal muscle and immunoblotting demonstrates a reduction in its molecular weight. Overlay assays show that -dystroglycan still has some residual laminin -2 binding. This is the first description of mutations in the human *LARGE* gene; we propose to name this novel form of congenital muscular dystrophy MDC1D.

**Identification of a locus for type I punctate palmoplantar keratoderma on chromosome 15q22-24.** A. Martinez-Mir<sup>1</sup>, A. Zlotogorski<sup>2</sup>, D. Londono<sup>3</sup>, D. Gordon<sup>3</sup>, A. Grunn<sup>4</sup>, E. Uribe<sup>5</sup>, L. Horev<sup>2</sup>, I.M. Ruiz<sup>5</sup>, N.O. Davalos<sup>5</sup>, O. Alayan<sup>2</sup>, J. Liu<sup>4</sup>, T.C. Gilliam<sup>4</sup>, J.C. Salas-Alanis<sup>6</sup>, A.M. Christiano<sup>1,7</sup>. 1) Department of Dermatology, Columbia University, New York, NY; 2) Department of Dermatology, Hadassah Medical Center, Jerusalem, Israel; 3) Laboratory of Statistical Genetics, Rockefeller University, New York, NY; 4) Columbia Genome Center, Columbia University, New York, NY; 5) Hospital General de Occidente Zapopan, Jalisco, Mexico; 6) Departamento de Dermatología, Servicio Médico Universidad Autónoma de Nuevo León, Monterrey N.L., Mexico; 7) Department of Genetics and Development, Columbia University, New York, NY.

The identification of the molecular basis of disorders of keratinization has significantly contributed to our understanding of skin biology, revealing new information on key structures in the skin such as the intermediate filaments, desmosomes and gap junctions. Among these disorders, there is an extraordinarily heterogeneous group known as palmoplantar keratodermas (PPK), for which only a few molecular defects have been described. Here we report the identification of the first locus for type I punctate PPK. A genomewide scan was performed on an extended autosomal dominant pedigree and linkage to chromosome 15q22-24 was identified. With the addition of two new families with the same phenotype, we confirmed the mapping of the locus for punctate PPK to a 9.98-cM interval, flanked by markers D15S534 and D15S818 (maximum two-point LOD score of 4.23 at  $\alpha=0.01$  for marker D15S988). According to the physical maps at NCBI (build #30), UCSC (April 2003 release), and deCODE Genetics, the critical linkage interval identified here corresponds to a region of 7.7 Mb, syntenic to mouse chromosome 9. These findings will facilitate the identification of a new gene involved in skin integrity that could also contribute to the understanding of the multiple organ involvement in the syndromic forms of PPK.

**Identification and characterization of a causal gene responsible for severe developmental delay and a pharyngeal anomaly from a patient with *de novo* translocation [46, XY, t(6;12)(q16;p12)].** K. Yamada<sup>1</sup>, T. Ono<sup>1</sup>, Y. Yamada<sup>1</sup>, N. Ishihara<sup>1,2</sup>, T. Ohki<sup>3</sup>, K. Miura<sup>3</sup>, T. Kumagai<sup>3</sup>, S. Sonta<sup>1</sup>, N. Wakamatsu<sup>1</sup>. 1) Dept Genet, Inst Developmental Res, Aichi Human Serv Ctr, Aichi; 2) Dept Pediatr, Nagoya Univ, Sch Med, Nagoya,; 3) Dept Pediatr Neurol, Central Hosp, Aichi Human Serv,Ctr, Aichi, Japan.

We report the identification and characterization of a gene located at a *de novo* balanced reciprocal translocation 46,XY,t(6;12)(q16;p12) in a patient with severe developmental delay and a pharyngeal anomaly. Fluorescent *in situ* hybridization (FISH) analysis demonstrated that a breakpoint in chromosome 6q16 to be located within a single BAC clone of 98I9 and that of chromosome 12p12 between 684O24 and 841C19. Southern blot analysis using cDNAs or genomic DNA fragments within these BAC clones as a probe as well as direct sequencing analysis of the PCR products between the two chromosomes demonstrated that a breakpoint at 6q16 is 9-kb upstream of *DKFZp564B0769* and that of 12p12 is intron 16 of *PEPP2* encoding phosphoinositol 3-phosphate-binding protein-2, respectively. The *PEPP2* consists of 26 exons containing multifunctional domain of Pleckstrin homologous domain which bind inositol phosphate and various proteins, and is expressed ubiquitously. These results suggest that *PEPP2* is important for normal brain development, and haploinsufficiency of the *PEPP2* likely resulting in severe developmental delay.

***RECQL4* mutation saving the helicase domain results in the RAPADILINO syndrome.** H.A. Siitonen<sup>1</sup>, O. Kopra<sup>1</sup>, H. Kääriäinen<sup>2</sup>, H. Haravuori<sup>1</sup>, R.M. Winter<sup>3</sup>, A-M. Säämänen<sup>4</sup>, L. Peltonen<sup>1,5</sup>, M. Kestilä<sup>1</sup>. 1) National Public Health Institute, Helsinki, Finland; 2) The Family Federation of Finland, Helsinki, Finland; 3) Institute of Child Health, London, UK; 4) University of Turku, Finland; 5) University of Helsinki, Finland.

RAPADILINO syndrome is a rare recessive syndrome with multiple dysmorphic features. Patients have a proportionate small stature, absent or hypoplastic thumbs, radius and patellae. They have characteristic face, normal intelligence and juvenile diarrhea that subsides later in life. RAPADILINO and Rothmund-Thomson syndromes (RTS) show some overlapping features such as radial ray defects but the hallmark symptom of RTS, poikiloderma is never seen in RAPADILINO patients. In contrast to dramatic dermatological changes leading to poikiloderma in RTS, the only dermatological sign in some RAPADILINO patients are patches of skin resembling caf-au-lait spots. Because mutations in *RECQL4* have been found in RTS, *RECQL4* was seen as an excellent candidate gene for RAPADILINO syndrome.

Linkage analysis was performed using markers D8S1836, D8S373 and D8S1925 flanking *RECQL4*, and significant lod scores were observed supporting this hypothesis. We identified four novel mutations in the *RECQL4* and the location of the consequent changes in the protein seems to determine either RAPADILINO or RTS phenotype. Mutations in genes of *RECQL* family are so far associated with Rothmund-Thomson, Werner and Bloom syndromes, all characterized by high predisposition to malignancies, RTS being especially associated with osteosarcoma. However, the most common RAPADILINO mutation, saving the helicase domain of the *RECQL4* protein, results in a non-cancerous phenotype. In situ hybridizations of mouse tissues revealed the most intense signal of *Recql4* in chondrocytes and enterocytes well reflecting the tissue symptoms of RAPADILINO. The high expression in chondrocytes will give a new insight to the normal bone development and growth disturbances in RAPADILINO as well as to the molecular mechanisms behind osteosarcoma, characteristic to RTS.

**Charcot-Marie-Tooth type 2D in a large Australian family of English origin is associated with a novel (H418A) mutation in the glycyl tRNA synthetase gene.** *N. Sambuughin<sup>1</sup>, K. Christodoulou<sup>2</sup>, H. Holley<sup>1</sup>, M. Tsingis<sup>2</sup>, K. Sivakumar<sup>1</sup>, L. Goldfarb<sup>3</sup>, G. Nicholson<sup>4</sup>.* 1) Neurology Research, Barrow Neurological Inst, Phoenix, AZ, US; 2) The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus; 3) National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, US; 4) ANZAC Medical Research Institute, Concord Hospital, New South Wales, Australia.

Charcot-Marie-Tooth (CMT) disease is a heterogeneous group of hereditary peripheral neuropathies. On the basis of electrophysiological and histopathological studies CMT had been divided into demyelinating (CMT1) and axonal (CMT2) types. Teenage-onset hereditary axonopathy with atrophy of thenar and interossei muscles, mild weakness of foot dorsiflexion developing later in the course of illness, and minimal subjective loss of sharp and vibration sense in the feet was observed in eight members of a four-generation Australian family of English origin. EMG revealed chronic partial denervation; nerve conduction studies showed mild slowing of motor conduction velocity (median motor range 44-50 m/sec, peroneal motor 43 m/sec to not obtainable). The condition in this family is linked to 7p14 chromosomal region, and the affected individuals share a 7-marker disease-associated haplotype. Two syndromes, an axonal type of CMT (CMT2D) and a distal form of spinal muscular atrophy (dSMA-V) recently reported to be linked to the 7p14 region, are caused by mutations in the glycyl tRNA synthetase (GARS) gene (*Am J Hum Genet* 72:1293-99, 2003). Analysis of GARS gene sequences in patients from the Australian family identified a novel heterozygous mutation changing His to Arg at a highly conserved residue 418 located between the core catalytic and the anti-codon binding domains of the GARS protein. The H418A mutation co-segregates with the disease in the family and is absent in 100 unrelated Caucasian controls. These findings further support the association between the CMT2D/dSMA type V syndrome and mutations in GARS. The mechanisms by which mutations in the ubiquitously expressed housekeeping glycyl tRNA synthetase cause these highly specific phenotypes remain to be elucidated.

**Characterization of the PSTPIP1-mediated Inflammatory Pathway Involved in PAPA Syndrome, an Autoinflammatory Disease.** *C.A. Wise<sup>1</sup>, E.J. Smith<sup>1</sup>, L.B. Bennett<sup>2</sup>, D. Zhang<sup>1</sup>, G. Wood<sup>3</sup>, D.L. Kastner<sup>3</sup>, V.M. Pascual<sup>2</sup>.* 1) Seay Research Center, Texas Scottish Rite Hosp, Dallas, TX; 2) UT Southwestern Medical School and Baylor Institute for Immunology Research, Dallas, TX; 3) Genetics and Genomics, NIAMS, NIH, Bethesda, Maryland.

PAPA (pyogenic arthritis, pyoderma gangrenosum, and acne, MIM 604416) syndrome, a new member of the heritable autoinflammatory disorders, is characterized by invasion of polymorphonuclear leukocytes into joints and skin, producing early-onset, destructive arthritis with variable skin involvement. We have shown that this disease is caused by missense mutations in the adaptor protein proline serine threonine phosphatase-interacting protein 1 (PSTPIP1, also known as CD2BP1), and that these mutations result in altered binding to PEST type protein tyrosine phosphatase (PTP PEST) and pyrin. Mutations in pyrin cause Familial Mediterranean Fever (FMF), an autoinflammatory disorder also marked by episodic influx of polymorphonuclear leukocytes into affected areas. We have hypothesized that PAPA syndrome is the result of an alteration in innate immunity in which affected individuals exist in a constitutive pro-inflammatory state, and that inflammation results from activation of a pyrin-mediated pathway downstream of PSTPIP1. To investigate this we have used expression profiling to characterize leukocytes of PAPA syndrome patients. Total cellular RNA from peripheral blood mononuclear cells of 9 affected individuals was compared to the same from 14 healthy controls using oligonucleotide microarrays. Parametric statistical group comparisons revealed 7 of transcripts differentially expressed between the two groups ( $p < 0.05$ ). These included PTP PEST and caspase 1, members of the pyrin pathway. Hierarchical clustering spontaneously differentiated PAPA affected individuals, with a range of clinical severity, from controls. Eleven transcripts remained significantly upregulated after stringent Bonferroni multiple testing correction, including genes implicated in apoptosis and monocyte activation. Further analyses of these data are in progress, as are experiments to identify members of the autoinflammatory pathway upstream of PSTPIP1.

**Genetic heterogeneity for Pyridoxine Dependent Seizures.** *C. Bennett*<sup>1</sup>, *H. Huynh*<sup>1</sup>, *I. Glass*<sup>1</sup>, *S. Gospe*<sup>2</sup>. 1) Division of Genetics & Development, RR-249, University of Washington School of Medicine, Seattle, WA; 2) Division of Pediatric Neurology, CHRMC 5D-4, Seattle, WA.

Pyridoxine-Dependent Seizures (PD) is a rare autosomal-recessive disorder where patients are dependent on high daily supplements of Vitamin B6 (Pyridoxine) to prevent seizures that are otherwise refractory to standard medication. Pyridoxine is a cofactor for glutamic acid decarboxylase (GAD), which converts glutamate to GABA. Examination of a PD patient brain sample, who died during seizure, revealed high levels of glutamate and low levels of GABA. High levels of glutamate and low levels of GABA were subsequently observed in the CSF of a PD patient prior to treatment, normalizing after administration of Pyridoxine. These clinical observations have led to the hypothesis that mutations within GAD cause PD. Several genetic studies have focused on the two isoforms of GAD, GAD65 and GAD67. One study examined three non-consanguineous North American families and excluded linkage to GAD65 by haplotype segregation analysis. These results were confirmed in similar genetic studies in Japanese PD patients where haplotype segregation analysis and mutational analysis excluded GAD65 and GAD67. In 2000, a genomewide linkage scan utilizing five families of North African descent (four of which were consanguineous) mapped a locus for PD at chromosome 5q31. A combined LOD score of 8.43 was obtained, and recombinant haplotype analysis defined a candidate interval of 5cM. Using DNA from 3 non-consanguineous North American PD families, we performed linkage analysis with markers within this 5q31 PD interval. Two families were consistent with linkage to this locus. However, recombination between PD and 5q31 markers was present in the third family, establishing genetic heterogeneity for PD. We hypothesize that mutations of an as yet undiscovered pyridoxine transporter gene may be the cause of PD. *GAD65GAD67*.

**Experience of a French network focused on the delineation of ceroid lipofuscinoses variants.** *C. Caillaud, and the French network on NCL (INSERM/AFM rare disorders).* Department of Genetics, Cochin Institute, Paris, France.

Neuronal ceroid lipofuscinoses (NCL) are neurodegenerative disorders, transmitted as an autosomal recessive trait, characterized by the accumulation of autofluorescent lipopigments, particularly in neurons. The clinical heterogeneity of these diseases (four main clinical forms and numerous variants) is clearly linked to the genetic diversity. Indeed, at least seven different loci have been described to date, among which six have been cloned : two encode an enzymatic protein and four a transmembrane protein of unknown function. An interactive network including neuropaediatricians, anatomopathologists, geneticists and researchers in France and United Kingdom was created in order to delineate the different forms of ceroid lipofuscinoses among French patients. The first step was the recruitment and clinical analysis of NCL patients. This work was performed by physicians who have an extensive experience of these pathologies, with the aim to define the various clinical presentations. Patients were first tested for the common CLN1, CLN2 and CLN3 genes at the enzymatic and/or molecular level and then for alternative genes (CLN6,...). Patients which do not map to any of the known NCL loci and may represent specific variants were collected. They will be used for the characterization of novel loci after morphological confirmation (presence of characteristic lipopigments) and distribution among homogeneous groups according to clinical, ultrastructural and geographic criteria. Our work demonstrated that the diversity of NCL clinical forms was linked not only to the presence of different mutations in the same gene, but also to the high number of genes involved in the pathogenesis of these diseases. Delineation of the clinical variants facilitates the genetic counselling of at-risk couples. Thus, since the beginning of the project, around twenty prenatal diagnoses have been performed in NCL families. This work will also permit to obtain informations on the pathophysiology, epidemiology and genetics of NCL and to open the way to the future development of specific therapies, including gene therapy.

**Molecular modeling of novel mutations in Fabry disease.** *K. Azibi<sup>1,2</sup>, C. Caillaud<sup>1,2</sup>, M.F. Szajnert<sup>2</sup>, J. Dussau<sup>1</sup>, J.P. Puech<sup>1</sup>, L. Poenaru<sup>1,2</sup>.* 1) Laboratoire de Genetique, Faculte de Medecine Cochin, Universite Paris 5, Paris, France; 2) Institut Cochin, Unite 567 INSERM, CNRS, Paris, France.

Fabry disease is an X-linked glycosphingolipid storage disease resulting from deficient activity of the lysosomal alpha-galactosidase A. 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. The alpha-galactosidase gene (GLA) was amplified and completely sequenced in 50 patients and 28 potential carriers from 67 families. 36 mutations (17 novel and 19 previously described) were identified. The novel mutations were : four nonsense mutations (Q212X, W245X, W349X and W262X), seven missense mutations (A156N, S297C, P265R, I354K, G361A, D264A and Q279H), a 1-bp insertion (InsG10680) and five deletions (1236del15, 5113delA, 10594delAT, 7317delA, 7338delG). These results confirm the previously reported heterogeneity of mutations causing Fabry disease and have also permitted to clarify the carrier status of females and to offer them reliable genetic counselling. To understand the structural basis of Fabry disease, we mapped the mutations described above onto a three-dimensional model of human alpha-galactosidase A (alpha-GAL). This model was based on the X-ray structure and the similarity of the chicken alpha-N-acetylgalactosaminidase, an enzyme which is closely related to alpha-GAL. Different programs were used to generate representations of protein topography (schematic topogram, alpha-helices and beta-strands, electronic density representations). They permitted to reveal the positions where mutations were located and the effects of these mutations on the active site of the enzyme and on the stability of the folded protein. This study allowed to define the severity of the mutations leading to Fabry disease and therefore to improve the phenotype prediction. Reference : S.C. Garman, D.N. Garboczi. Structural basis of Fabry disease. *Mol. Genet. Metab.* 2002, 77, 3-11.

**Molecular analysis of the glomulin gene in glomuvenous malformation families.** *M.T. Kloos<sup>1</sup>, E. Crocker<sup>1</sup>, C. Haisley-Royster<sup>2</sup>, C.L. Hunter<sup>3</sup>, T.P. Leedom<sup>1</sup>, N.C. Nevin<sup>4</sup>, S.J. Orlow<sup>5</sup>, N. Prose<sup>2</sup>, S.A. Simpson<sup>6</sup>, G.H. Vance<sup>3</sup>, D.F. Murrell<sup>7</sup>, D.A. Marchuk<sup>1</sup>.* 1) Dept. of Mol. Genet. and Microbiol; 2) Dept. of Dermatology, Duke University Medical Center, Durham, NC; 3) Dept. of Med. and Mol. Genet., Indiana University, Indianapolis, IN; 4) Northern Ireland Regional Genetics Centre, Belfast, Northern Ireland; 5) Dept. of Dermatology, NYU School of Medicine, New York, NY; 6) Dept. of Med. Genet., Aberdeen Royal Hospitals, Aberdeen, Scotland; 7) St. George Hospital, Sydney, Australia.

Mutations in the gene encoding glomulin (also termed FAP68) have recently been reported in patients with autosomal dominant glomuvenous malformations (GVMs). GVMs are characterized by vascular abnormalities involving smooth-muscle-like glomus cells, which present as cutaneous bluish-purple lesions. We ascertained and collected DNA for seven GVM families/patients from the U.S., U.K., and Australia. Five were large enough to either establish linkage or a common disease haplotype near the glomulin locus on chromosome 1p22. Mutations were identified in glomulin in all seven families, two of which are novel. All are predicted to be truncating mutations. In 4 of the 7 families/patients a 157delAAGAA was identified. This mutation has been previously described and with this report, identified in 11 of 27 families described to date. This common mutation was previously attributed to a founder effect in families collected from diverse geographical areas. We also find that our 4 families with this mutation show some evidence of a common disease haplotype with each other, and possibly with those previously reported. If this is due to a common ancestral founder, it suggests a relatively ancient origin for this mutation, as it has now been identified in families from 8 countries on 3 continents. Using existence sequence databases, we identified a novel coding exon which was not previously recognized in the original report. This exon was shown to be an authentic part of the glomulin transcript by RT-PCR analysis from a panel of adult human tissues. No mutations were found to date in this novel exon, but it should be included in subsequent mutation screening to ensure complete coverage of the gene.

**Homozygosity Mapping of a Gene for Arterial Tortuosity Syndrome.** *P. Coucke<sup>1</sup>, M.W. Wessels<sup>2</sup>, P. Van Acker<sup>1</sup>, R. Gardella<sup>3</sup>, S. Barlati<sup>3</sup>, P.J. Willems<sup>4</sup>, M. Colombi<sup>3</sup>, A. De Paepe<sup>1</sup>.* 1) Dept Medical Genetics, University Hospital Ghent, Ghent, Belgium; 2) Dept Clinical Genetics, Obstetrics and Gynecology, Erasmus University, Rotterdam, The Netherlands; 3) Dept of Biomedical Sciences and Biotechnology, University of Brescia, Italy; 4) Synergene, Belgium.

Arterial tortuosity syndrome (ATS, MIM 208 050) is a rare connective tissue disorder characterized by generalized tortuosity, elongation, stenosis and aneurysms of the major arteries. Skin and joint abnormalities including hyperextensibility or hyperlaxity of the skin, joint laxity or contractures and inguinal hernias, reminiscent of other connective tissue diseases, can also be observed. Other phenotypic abnormalities include micrognathia, elongated face, high palate, beaked nose, sliding hernia and ventricular hypertrophy. Histopathologic studies show abnormalities of the elastin network in the large arteries. The mode of inheritance of ATS is autosomal recessive, but the disease gene has not yet been identified. Until now, a very limited number of ATS patients have been described and few multiplex families have been published. However, some inbred multiplex families originating from Morocco and Italy have recently been reported and create the opportunity to localize the ATS gene. We have performed a total genomesearch by homozygosity mapping in three consanguineous multiplex families, and mapped the ATS gene to chromosome 20q13. Recombinations within an extended haplotype of 11 microsatellite markers localized the ATS gene between markers D20S836 and D20S109, an interval of 9.5 cM which represents 4.1 Mb on the physical map. Based on the different databases, the candidate region for ATS contains 31 predicted transcripts. Genes or homologues of structural genes involved in the extracellular matrix, more in particular those involved in the elastogenesis pathway, will be considered as strong candidates. Until now, no obvious candidate gene has been identified.

**DHPLC screening of ATP8B1 in Finnish women suffering from Intrahepatic Cholestasis of Pregnancy.** *J.N.*

*Painter<sup>1</sup>, M. Savander<sup>1</sup>, A-E. Lehesjoki<sup>1</sup>, A. Ropponen<sup>2</sup>, K. Aittomki<sup>1</sup>.* 1) Folkhlsan Institute of Genetics, Biomedicum P.O. Box 63, Department of Medical Genetics, University of Helsinki 00014 Finland; 2) Department of Gynaecology and Obstetrics, Helsinki University Hospital, 00029 Helsinki, Finland.

Intrahepatic Cholestasis of Pregnancy (ICP, also known as obstetric cholestasis) is a disease occurring usually in the third trimester of pregnancy, characterised by generalised (and possibly intense) itching and increased levels of total bile acids in maternal blood. Whilst the condition can be particularly uncomfortable for the mother, the symptoms resolve spontaneously after delivery. The outcome for the foetus is less certain, as ICP is associated with increased rates of premature birth, intrauterine distress and/or sudden death. Whilst the aetiology of the disease is still largely unclear, and likely to be genetically heterogenous, ICP has been reported in mothers of children affected with progressive familial intrahepatic cholestasis (PFIC) types 1, 2 and 3. One of the cholestasis genes, ATP8B1, causing both PFIC1 and benign recurrent intrahepatic cholestasis (BRIC), has often been implicated as a possible cause of ICP. The risk for women heterozygous for mutations in ATP8B1 may be increased, as mothers of children suffering from both diseases, relatives of BRIC patients as well as BRIC sufferers themselves, may experience ICP. In addition, BRIC is at least superficially similar to ICP, as cholestatic episodes are periodic, of variable intensity, apparently 'benign' with no permanent liver damage, and resolve after some time. To investigate whether ATP8B1 could be a cause of ICP in Finland we are using denaturing high-performance liquid chromatography (DHPLC) to screen affected women for mutations in the 27 exons of ATP8B1. At the time of writing we have screened 144 sporadic and 19 familial patients. Despite finding a number of intronic and exonic SNPs, some apparently unique to the Finnish population, no disease causing mutations have as yet been discovered. At present, it appears that ATP8B1 is not responsible for ICP in Finland.

**Unexpected 11p15 anomalies in Sotos syndrome and NSD1 mutations in Beckwith-Wiedemann syndrome.** G. BAUJAT<sup>1</sup>, M. RIO<sup>1</sup>, D. SANLAVILLE<sup>1</sup>, A. MUNNICH<sup>1</sup>, C. GICQUEL<sup>2</sup>, V. CORMIER-DAIRE<sup>1</sup>, L. COLLEAUX<sup>1</sup>. 1) INSERM U393, Hopital Necker-Enfants Malades, Paris, FRANCE; 2) Laboratoire d'Explorations Fonctionnelles Endocriniennes et Inserm U515, Hpital Armand Trousseau, Paris, France.

Sotos syndrome is an overgrowth syndrome characterized by pre- and postnatal overgrowth, macrocephaly, advanced bone age, variable degree of mental retardation and typical facial features. Deletions and point mutations of the NSD1 gene account for largely 80% of Sotos syndrome, while the disease mechanism of other cases remains unknown.

On the other hand, Beckwith-Wiedemann syndrome (BWS) is a distinct overgrowth condition characterized by macroglossia, omphalocele, visceromegaly, hypoglycemia and an elevated risk of developing embryonal tumors. BWS is a genetically heterogeneous disorder caused by dysregulation of imprinted growth regulatory genes within the 11p15 region. CDKN1C mutation and 11p15 uniparental disomy account for 30% of patients but in many cases the disorder is due to epigenetic defects i.e. isolated hypermethylation of H19 or demethylation of KCNQ1OT. The molecular defect underlying a significant proportion of sporadic BWS cases remains unknown.

Although Sotos and BWS are clinically distinct conditions, they share common features such as macrosomia, neonatal hypoglycemia, seizure or cardiac anomalies. Based on this phenotypic overlap, we tested whether unexplained Sotos cases could be related to 11p15 anomalies and whether unexplained BWS could be related to NSD1 deletions or mutations. Indeed, one UPD and one imprinting mutation of the 11p15 region were identified in a series of 19 Sotos patients carrying no NSD1 anomaly. On the other hand, 51 BWS children with no 11p15 anomaly were tested for NSD1 mutation. No deletion was found but most interestingly, we identified 2 mutations in 11 children. These results suggest that, despite clinical differences, the two disorders may have more similarities than previously thought, based on molecular genetic findings and we propose that NSD1 protein could be involved in imprinting of the chromosome 11p15 region.

**Evaluation of the Multiplex Ligation-Dependent Probe Amplification (MLPA) technology in the diagnosis of Rett syndrome.** *S. Boulanger, L. Mariage, L. Van Maldergem, Y. Gillerot, P. Hilbert.* Human Genetics Center, Institute of pathology and Genetics, Loverval, Belgium.

Rett Syndrome (RTT) is an X-linked progressive neurodevelopmental disorder and one of the most common causes of mental retardation in females, with an incidence of 1 in 10.000-15.000. Sequencing of the coding and splicing regions of the methyl-CpG binding protein (MECP2) gene is thought to yield a 50 to 80 % range of disease-causing mutations considering patients fulfilling clinical criteria. Recently, gross rearrangements of the MECP2 gene, which were not evidenced by classical sequencing, were detected by Southern blot analysis. It could represent a significant proportion of cases lacking mutations. On a total of 116 girls selected with a panel of signs and symptoms compatible with RTT, we have identified 22 classical mutations. In order to evaluate the importance of possible gross rearrangements in the mutation-negative cases, we used Multiplex Ligation-Dependent Probe Amplification (MLPA) technology. Up to now, 53 of the negative cases were tested by MLPA and 4 showed a deletion of one or more exons. Two of them have deletion of exons 3 and 4, one a deletion of the first exon and the last one a deletion of exon 4. For two patients (one with deletion of exon 4 and one with deletion of exons 3 and 4), we confirmed the rearrangement by Southern blot analysis. These preliminary results confirm that a search for MECP2 deletions is important for the diagnosis of Rett Syndrome and that MLPA is a simple and fast technology to reveal these alterations.

**Animal models for Gdi1 non-specific mental retardation.** P. D'Adamo<sup>1</sup>, H. Welzl<sup>1</sup>, D. Wolfer<sup>1</sup>, H.P. Lipp<sup>1</sup>, L. Rinaldi<sup>2</sup>, D. Toniolo<sup>2</sup>. 1) Anatomy, University of Zurich, Zurich, Switzerland; 2) DIBIT, San Raffaele Scientific Institute, Milano, Italy.

MR is a common and highly heterogeneous human disorder characterized by childhood onset and mental handicap as the only clinical symptom. Many studies have been undertaken to determine the causes of MR and all have demonstrated high heterogeneity due to both genetics and environmental factors. In recent years a small number of X linked genes for MR (MRX) were identified and they confirmed the heterogeneity of the disorder and demonstrated that the newly identified genes are important tools for understanding the molecular basis of MR. One of the genes identified was GDI1, which encodes one of the proteins controlling the activity of the small GTPases of the Rab family, in vesicle fusion and intracellular trafficking. To gain insights into the role of the genes identified in MR we have produced a mouse mutant of Gdi1. The Gdi1 deficient mice were viable and fertile. They were normal in many tasks to assess learning capacity and emotional behavior, but they were impaired in tasks requiring formation of temporal associations suggesting defects in short term memory. They also show lowered aggression and altered social behavior. Our results show that in mice, as in humans, lack of Gdi1 spares most CNS functions and affects a hippocampus function related to short term memory formation, suggesting evolutionary conservation of basic cognitive function(s) and tentatively confirming that simple animal models can be of great value in the study of human MR. Moreover, lack of Gdi1 was not responsible for alterations of parameters of SV exocytosis and neurotransmitter release. Electrophysiological analysis of the Gdi1 KO mice demonstrated that lack of Gdi1 is responsible for specific deficits in short and long term synaptic plasticity, suggesting that the mice could present depletion of the reserve synaptic vesicle pool. This phenotype was confirmed using electron microscopic studies of the Gdi1 KO hippocampus showing that the number and the distribution of SV is altered in the KO.c.

**Comparison of phenotypes, neuronal electrophysiology, and gene expression patterns between Fmr2 and Af5q31 KO mice.** *Y. Gu*<sup>1</sup>, *B. Antalffy*<sup>2</sup>, *E. Weeber*<sup>3</sup>, *D. Armstrong*<sup>2</sup>, *D.L. Nelson*<sup>1</sup>. 1) Molec & Human Genetics, Baylor College Med, Houston, TX; 2) Department of Pathology, Baylor College of Medicine, Houston, Texas 7703; 3) Division of Neuroscience, Baylor College of Medicine, Houston, Texas 7703.

Fmr2 and AF5q31 belong to the AF4/FMR2 gene family sharing several highly homologous domains with two other members of this family: AF4 and LAF4. In humans, loss of function of FMR2 is associated with mild mental retardation; expansion and methylation of a CCG triplet repeat at FRAXE is the most common mutation. No phenotypes associated with loss of function of other members in this family have been described in humans. Available evidence from vertebrate and fruit fly studies suggest that this family is involved in gene expression regulation, cell expansion, and embryonic development as well as adult organ function. We are interested in studying these functions through development of mouse models. We have created both Fmr2 and AF5q31 knockout models and studied these two models through analysis of phenotypes, electrophysiological responses in neurons of the hippocampus, targeted gene expression patterns and neuronal expansion. The results showed that absence of either gene affected electrophysiological responses in neurons of hippocampus and target gene expression in a distinct way. For example, Af5q31 knockout mice exhibited abnormality in paired pulse facilitation, but normal long term potentiation of hippocampal neurons while the Fmr2 knockout displayed normal paired pulse facilitation, with abnormal long term potentiation. In AF5q31 knockout mice, we found at least 50% embryonic lethality of offspring. Embryonic death can occur as early as day 12.5 post coitus (PC). Other knockout embryos appeared developmentally delayed compared with normal control embryos at the same stage. In Fmr2 knockout mice, GABA neurons were reduced in the cerebrum, especially in the hippocampus. Expression of members of the Sp family was affected in both models and the results demonstrated different members of the Sp family were regulated by FMR2 and AF5q31.

**Novel *MECP2* mutations identified from Japanese patients with classical and variant Rett syndromes. Y.**

*Yamada*<sup>1</sup>, *K. Miura*<sup>2</sup>, *T. Kumagai*<sup>2</sup>, *T. Fujii*<sup>3</sup>, *T. Ishikawa*<sup>4</sup>, *T. Tandou*<sup>5</sup>, *N. Nomura*<sup>1</sup>, *K. Yamada*<sup>1</sup>, *S. Sonta*<sup>1</sup>, *N. Wakamatsu*<sup>1</sup>. 1) Dept Genetics, 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, Shiga Med Ctr Child, Moriyama, Shiga, Japan; 4) Dept Pediatr, Med Sch, Nagoya City Univ, Nagoya, Aichi, Japan; 5) Dept Pediatr, Kanoiwa Hosp, Koufu, Yamanashi, Japan.

The Rett syndrome (RTT, MIM 312750), a progressive neurodevelopmental disorder, is caused by mutations in the methyl-CpG-binding protein 2 gene (*MECP2*) located at Xq28, as X-linked dominant trait. We have been analyzing the gene in Japanese patients divided into classical RTT, variant RTT, and mentally retarded patients with Rett-like features. Mutations in *MECP2* have been identified in most of the patients with classical and variant RTT, and 70 % of all the mutation were common mutations and other 30 % were rare mutations (Yamada et al, Hum Mut, 2001, 18: 253). By the additional studies to confirm the diagnosis of RTT using the PCR method coupled with direct sequencing, we report here four novel *MECP2* mutations responsible for classical and variant RTT. In a variant RTT patient, two single base substitutions, a novel C to G transversion resulting in amino acid change of F157L and a C to T transition generating A201V reported as a non-pathogenic variant, were found. The missense mutation F157L is consider to be associated with the clinical phenotype, since that was located at the methyl-binding domain and some other causal mutations were detected around the site. A nonsense mutation (E137X) generated from a nucleotide substitution of G to T, and two deletion mutations, 119delAG and 855delA resulting in amino acid frame-shift, 40fs48X and 285fs288X, respectively, are also new to the data base of *MECP2* mutations. The patient with 855delA had also the A201V in the same allele. Furthermore, in a patient with the most common mutation (T158M), a new missense mutation G232A raised from a G to C substitution was also detected. However, the G232A seems to be a new non-pathogenic variant, since the substitution was also found in the father's X chromosome.

Program Nr: 2373 from 2003 ASHG Annual Meeting

**Studies of FMRP-binding RNAs with a yeast tri-hybrid system.** *A. Wronska, W. Ju, W.T. Brown, N. Zhong.*  
Department of Human Genetics, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY.

Fragile X syndrome is the most common inherited mental retardation resulted from lack of the fragile X gene FMR1-encoded protein FMRP, which function has been suggested to regulate cellular translations through binding to 4-5% of RNA molecules. In this study, we have employed an in vivo approach to test FMRP binding to a certain number of RNA molecules that have been determined to be differentially transcribed in fragile X cells and to identify FMRP-binding RNAs. A human FMR1 coding sequence was cloned to a yeast tri-hybrid system to express FMRP as a bait protein, for interacting with prey RNAs. Eight target RNA molecules, four G-quartet regions and four non-G-quartet regions were selected from the MAP1B, NAP22, TMEM1, UNC13, and were tested for the FMRP-RNA binding. Our results showed that all RNA sequences with G-quartet structure bind to FMRP, but all sequences with non-G-quartet did not bind to FMRP. In addition, four novel RNA molecules, the protein tyrosine kinase (NTRK2), toll/interleukin-1 receptor-like (TLL3), G-protein-coupled receptor 45 (GPR45), and a hypothetical transmembrane protein (SBB154), were identified from library screening to bind to FMRP when a human brain cDNA library was screened by FMRP.

**Mutations in ZNF41 are associated with cognitive deficits.** *S. Shoichet<sup>1</sup>, K. Hoffmann<sup>1</sup>, C. Menzel<sup>1</sup>, U. Trautmann<sup>2</sup>, B. Moser<sup>1</sup>, M. Hoeltzenbein<sup>1</sup>, B. Echenne<sup>3</sup>, H. vanBokhoven<sup>4</sup>, C. Moraine<sup>5</sup>, J.P. Fryns<sup>6</sup>, J. Chelly<sup>7</sup>, H.D. Rott<sup>2</sup>, H.H. Ropers<sup>1</sup>, V.M. Kalscheuer<sup>1</sup>.* 1) Max-Planck Institute for Molecular Genetics, Berlin, Germany; 2) Institute of Human Genetics, University of Erlangen-Nuremberg, Germany; 3) Centre Hospitalier Universitaire de Montpellier, Hopital Saint-Eloi, 34295 Montpellier Cedex 5, France; 4) Department of Human Genetics, University Medical Centre, Nijmegen, The Netherlands; 5) Services de Génétique -INSERM U316, CHU Bretonneau, Tours, France; 6) Center for Human Genetics, Clinical Genetics Unit, Leuven, Belgium; 7) Institut Cochin de Génétique Moléculaire, CNRS/INSERM, CHU Cochin 75014 Paris, France.

Non-syndromic X-linked mental retardation (MRX) is defined by an X-linked inheritance pattern of low IQ and problems with adaptive behaviour, and the absence of additional specific clinical features. The MRX genes identified to date account for less than one third of all MRX, suggesting that numerous other gene defects cause the disorder in other families. In a patient with severe non-specific mental retardation and a de novo balanced translocation t(X;7) (p11.3;q11.21), we have cloned the DNA fragment containing the X-chromosomal and the autosomal breakpoint. In silico analysis provided no indication for a causative role for the chromosome 7 breakpoint in MR, whereas on the X-chromosome the ZNF41 gene was found to be disrupted. Expression studies indicated that ZNF41 transcripts are absent in a cell line of the patient, suggesting that the mental disorder in this patient results from loss of functional ZNF41. Screening of a panel of MRX patients led to the identification of two potentially disease-causing ZNF41 mutations. A proline to leucine amino acid exchange is present in affected members of one MRX family. A second family harbours a mutation that results in loss of transcription of specific ZNF41 splice variants. Wild type ZNF41 contains a highly conserved transcriptional repressor domain that is linked to mechanisms of chromatin remodelling. Our results suggest that ZNF41 is critical for cognitive development; further studies aim to elucidate the specific mechanisms by which ZNF41 alterations lead to mental retardation.

**Enhanced exon skipping causing mild Börjeson-Forssman-Lehmann Syndrome results from a novel PHF6 mutation.** *D.J. Picketts<sup>1</sup>, D. Vallée<sup>1</sup>, M.A. Lazzaro<sup>1</sup>, A.G. Hunter<sup>2</sup>, G.E. Graham<sup>2</sup>.* 1) Molecular Medicine Program, Ottawa Health Research Inst, Ottawa, ON, Canada; 2) Childrens Hospital of Eastern Ontario, Ottawa, Ontario, Canada.

Börjeson-Forssman-Lehmann (BFL) syndrome is an X-linked recessive disorder characterized by moderate to severe mental retardation, obesity, epilepsy, hypogonadism, gynaecomastia, and minor facial anomalies including large ears and excessive facial fat. The disease was recently shown to result from mutations in the PHF6 gene located in Xq26. Here, we present mutational analyses for two Canadian BFL syndrome families. One family with classical features of BFL syndrome had a missense mutation in exon 8 (Arg257Gly) that has been previously reported. Affected individuals from the second family displayed a less severe phenotype including mild developmental delay. Analysis of the PHF6 gene in this family identified a T to A substitution in intron 2, creating a novel acceptor splice site 7 base pairs upstream of the consensus sequence. Inclusion of the seven nucleotides causes a frameshift and protein truncation. As such, we postulated that the mild phenotype might result from incomplete use of the aberrant splice acceptor site thereby creating some normally spliced PHF6 transcript. However, RT-PCR analysis demonstrated two aberrantly spliced products, a larger transcript (+7 bp) in which the novel acceptor splice site was used, and a smaller transcript in which exon 3 was skipped. Skipping of exon 3 maintains the ORF producing a protein that is reduced in size by 34 residues yet retains both the nucleolar localization signal and the two PHD zinc finger domains. To confirm that these transcripts are translated, in the absence of an antibody to the PHF6 protein, we used cell fractionation to isolate polysomes from patient lymphoblasts and demonstrated by RT-PCR that both altered transcripts are associated with ribosomes. In light of these results, we predict that the smaller PHF6 protein can functionally compensate for the loss of full-length PHF6 and that the mild disease is likely due to haploinsufficiency or reduced protein function due to the loss of a functional domain encoded by exon 3.

**Analysis of polymorphic sites within ornithine transcarbamylase (OTC) gene improves mutation analysis in females with OTC deficiency.** *L. Dvorakova*<sup>1</sup>, *L. Stolnaja*<sup>1</sup>, *E. Tietzeova*<sup>1</sup>, *L. Azevedo*<sup>2,3</sup>, *L. Vilarinho*<sup>4</sup>, *A. Amorim*<sup>2,3</sup>, *E. Hrubá*<sup>1</sup>, *M. Hrebicek*<sup>1</sup>. 1) Inst Inherit Metabol Disorders, Prague, Czech Republic; 2) Inst. Pathol. Immunol. of Univ. Porto, Porto, Portugal; 3) Faculty of Sciences, Univ. Porto, Porto, Portugal; 4) IGMJM, Porto, Portugal.

Ornithine transcarbamylase (OTC) deficiency is the most common urea cycle disorder. In spite of its X-linked inheritance increasing number of female patients manifesting this disorder is diagnosed. Four out of 16 Czech and Slovak probands, in whom the diagnosis was proved by mutation analysis, were females. Direct sequencing of PCR products from peripheral lymphocyte DNA revealed heterozygosity for missense (K210Q, G195R), nonsense (Q285X) and splicing (IVS7+1G>T) mutations. In other four patients (two males, two females) who had clinical and biochemical signs of OTC deficiency we were unable to find any mutation. One of these patients was a female with typical clinical and biochemical symptoms of OTC deficiency. As sequencing failed to identify any mutation, we analyzed six frequent intragenic polymorphisms that form 7 haplotypes in Czech population. Comparison of these polymorphisms and haplotypes in the patient and her parents suggests that the patient lacks a part of her father's OTC gene encompassing exon 10. X-inactivation analysis performed in DNA isolated from patient's peripheral leukocytes, salivary cells and urinary sediment cells using a method assaying differential methylation of HUMARA gene shows only slight preferential inactivation of maternal allele (70:30, 70:30 and 67:33, respectively). This case shows that the intragenic polymorphism and haplotype studies may significantly contribute to proper diagnosis in females heterozygous for X-linked disorders. Supported by grants NE 6557-3-01 from Ministry of Health and VZ 111100003 from Ministry of Education of the Czech Republic and by Portugal grants PRAXIS XXI/BD/21780/99 and POCTI/1999/MGI/35809.

**Identification of Novel Mutations in Filipino Maple Syrup Urine Disease Cases.** *C.L.T. Silao<sup>1,2</sup>, C.D. Padilla<sup>1,2</sup>, H. Nakamura<sup>3</sup>, M. Matsuo<sup>3</sup>*. 1) Institute of Human Genetics, National Institutes of Health, University of the Philippines, Manila; 2) Department of Pediatrics, University of the Philippines College of Medicine-Philippine General Hospital, Manila, Philippines; 3) Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan.

Maple Syrup Urine Disease (MSUD) is a rare autosomal recessive inborn error of metabolism caused by deficient activity of the branched chain ketoacid dehydrogenase (BCKAD) complex. This multienzyme complex catalyzes the oxidative decarboxylation of 3 branched chain ketoacids (BCKA) derived from the essential branched chain amino acids (BCAA) leucine, isoleucine, and valine. The eventual accumulation of these BCKAs and BCAAs produces the distinct burnt sugar smell noted in these patients and may eventually cause severe neurodegenerative or rapidly fatal consequences if left untreated. Several causal mutations have been identified in the 3 catalytic components comprising this enzyme complex. A study to determine the molecular basis of MSUD was carried out on two subjects belonging to unrelated families from the Philippines. Mutation analyses, consisting of polymerase chain reactions and direct sequencing of the entire dihydrolipoyl transacylase (E2) and the alpha subunit of the branched chain alpha-ketoacid decarboxylase (E1 alpha) gene of the BCKAD complex, yielded a novel missense mutation and a novel nonsense mutation respectively. These two novel mutations presented, one of which occurring homozygously, caused classical Maple Syrup Urine Disease. To our knowledge the compound heterozygous patient is the second known case, so far, having simultaneously two different mutations in 2 different genes of the BCKAD proteins. Molecular characterization of MSUD is essential to better understand this metabolic disorder. Our results also provides further evidence of genetic heterogeneity in Filipino MSUD patients.

**Cytochrome c oxidase deficiency due to mutations in SCO2 gene.** *K. Vesela<sup>1</sup>, H. Hansikova<sup>1</sup>, M. Tesarova<sup>1</sup>, P. Martasek<sup>1</sup>, J. Houstek<sup>2</sup>, J. Zeman<sup>1</sup>.* 1) Charles University, Prague, Czech Republic; 2) Academy of sciences, Prague, Czech Republic.

Cytochrome c oxidase (COX), terminal protein complex of mitochondrial respiration chain, plays an important role in the energy utilization of OXPHOS. Several proteins involved in cytochrome c oxidase (COX) assembly processes were described. One of them, Sco2 protein, encoded by SCO2 gene, transports Cu into COX subunits and enables COX to accept electrons from cytochrom bc. So far less than 15 children were described with mutations in SCO2 gene. Here we present the results of molecular analyses in six new children with mutations in SCO2 gene. **Material and Methods:** The DNA from tissue or fibroblasts of 30 children with isolated COX deficiency was examined. The coding region of SCO2 gene was sequenced and results were confirmed by RFLP. **Results:** Mutations in SCO2 gene were found in 6 children from 4 unrelated families. One child was compound heterozygote for the mutation C1280T (Q53X) and G1541A (E140K), five others were homozygotes for G1541A mutation. The course of the disease was fatal in all children, but the clinical symptoms differed significantly. Clinical symptoms in patients with homozygous G1541A mutations developed between the third and sixth month of age and included inspiratory stridor, hypotonia, encephalopathy and progressive respiratory insufficiency. On the contrary, fatal neonatal encephalopathy with severe hypertrophic cardiomyopathy was observed in the compound heterozygote. **Discussion:** COX deficiencies represent one of the most common mitochondrial diseases in childhood. Two distinct clinical phenotypes were recognised in our group of patients with COX deficiency and mutations in SCO2 gene. *Supported by GAUK 3/2002/c; VZ 111100003..*

Program Nr: 2379 from 2003 ASHG Annual Meeting

**Development of an ALSIN gene-targeted mouse model.** *H. Deng, R. Fu, H. Zhai, A. Azim, T. Siddique.* Neurology Dept, Northwestern Univ, Chicago, IL.

Amyotrophic lateral sclerosis (ALS) is a progressive paralytic disorder caused by degeneration of the upper motor neurons in the motor cortex and lower motor neurons in the brainstem and the spinal cord. We previously linked the recessive form of juvenile ALS type 3 (ALS2) to chromosome 2q3335, and subsequently we reported that a differentially spliced gene encoding short and long forms of a protein, which we named alsin, is mutated in both juvenile ALS and juvenile primary lateral sclerosis (JPLS). This gene has two transcriptional forms with two distinct poly(A) signals. The short form has 4 exons and the long form has 34 exons, respectively, with the first 4 exons shared by both forms. The gene products, which we called alsin, are two distinct proteins with the short one being 396 amino acids and long one being 1657 amino acids in size, and the short form shares 371 amino acids with the long form at the N-terminus (Yang et al, 2001). To investigate the physiological function of alsin and the pathogenic mechanism by which loss of alsin leads to ALS or PLS, we constructed a targeting vector that was designed to replace exon 4 and a part of exon 3 with neo- cassette. We identified two positive ES cells with right targeting events and alsin gene-targeted mice were developed from these ES cells. The homozygous mice are fertile and phenotypically normal by 4 months. The additional updated data will be presented.

**Screening to Develop a Zebrafish Model of Spinal Muscular Atrophy.** *A.Q. Hejmanowski<sup>1</sup>, M.L. McWhorter<sup>2</sup>, C.E. Beattie<sup>2</sup>, A.H.M. Burghes<sup>3</sup>, T.W. Prior<sup>1</sup>*. 1) Department of Pathology, Ohio State University, Columbus, OH; 2) Department of Neuroscience, Ohio State University, Columbus, OH; 3) Department of Molecular and Cellular Biochemistry, Ohio State University, Columbus, OH.

Spinal Muscular Atrophy (SMA) is a recessive disease that causes deterioration of anterior horn cells in the spinal column, leading to a loss of muscle innervation and muscle atrophy. It is the leading genetic cause of death among children under the age of two. SMA is caused by mutations in the Survival of Motor Neuron (SMN) gene. There are two copies of SMN located on chromosome 5: SMN1 (telomeric) and SMN2 (centromeric). Mutations in SMN1 cause disease. Several animals have genes homologous to SMN, including *Rattus norvegicus*, *Mus musculus*, *Danio rerio*, *Sus scrofa*, and *Bos taurus*. Given the size restraints of most labs, studies to find a disease model have focused on the first three animals. Zebrafish (*Danio rerio*) have only one copy of SMN, which means the event that caused humans to have two copies took place later in evolution. The small size, fast reproductive rate and SMN similarities have made the zebrafish a good model candidate. In addition, the developing zebrafish embryo is transparent, allowing close observation during critical developmental periods. Our work has focused on screening mutagenized zebrafish to locate a fish with disease causing mutations in SMN. We have worked with DNA from fish exposed to gamma irradiation and fish exposed to ENU. Gamma irradiation tends to cause large deletions or rearrangements. These fish are screened using fluorescent dosage on an ABI 377. 134 individual zebrafish have been screened using the 3' end of -actin and SMN exon 7. In our next round of gamma fish, we will be adding SMN exon 1. ENU tends to cause single base changes or single base deletions. These fish are screened using denaturing high performance liquid chromatography (DHPLC) on a Transgenomic WAVE system. 260 zebrafish have been DHPLC screened for all SMN exons except 5.

**Investigation of TGF- plasma levels as a modifier of the phenotype of Hereditary Hemorrhagic Telangiectasia.**

*T.L. Squire*<sup>1,4</sup>, *S. Srinivasan*<sup>1,4</sup>, *R.J. Akhurst*<sup>2</sup>, *H.M. Arthur*<sup>3</sup>, *D.A. Marchuk*<sup>1</sup>. 1) Dept. of Molecular Genetics & Microbiology, Duke University Medical Center, Durham, NC, USA; 2) University of California at San Francisco, San Francisco, CA, USA; 3) Institute of Human Genetics, University of Newcastle, Newcastle upon Tyne, NE1 3BZ, UK; 4) Both individuals contributed equally to this work.

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder characterized by multisystemic vascular dysplasia. Mutations in either of two genes, *ENDOGLIN* (*ENG*) or *ACTIVIN RECEPTOR-LIKE KINASE 1* (*ACVRL1* or *ALK1*), endothelial receptors for transforming growth factor beta (TGF-), are known to cause the disease. In humans, the expression of the clinical symptoms is highly variable, even among individuals carrying the same mutation, suggesting that additional genetic and/or environmental factors act as modifiers in the progression of this disease. One such modifier has been suggested for a mouse model of HHT type 1 (*Eng*<sup>+/-</sup> mice), where a higher frequency of vascular lesions was observed in the 129/Ola background than in C57BL/6 background. Since the 129/Ola strain contains low latent TGF-1 levels in plasma (median levels approximately 60% of that for C57BL/6), genes regulating TGF-1 levels were suggested as potential modifiers of the disease phenotype. To test this hypothesis, we crossed *Acvr11*<sup>+/-</sup> and *Eng*<sup>+/-</sup> mice separately with *Tgfb1*<sup>+/-</sup> mice, in a predominantly C57BL/6 genetic background. The single (*Acvr11*<sup>+/-</sup>) and double heterozygous offspring (*Acvr11*<sup>+/-</sup> *Tgfb1*<sup>+/-</sup>) were phenotyped at age 11 months by gross anatomical examination at necropsy. Greater than 50% of all *Acvr11* heterozygotes displayed a noticeable HHT phenotype, the strongest indicator of which was dilated sinusoidal vessels in liver. The incidence of the HHT phenotype was not substantially different between the *Acvr11* single and double heterozygotes. Additionally, preliminary investigation of 10 *Eng*<sup>+/-</sup> mice revealed no difference in the disease prevalence as a result of *Tgfb1* genotype. These results suggest that plasma TGF-1 concentration may not be an important modifier of the phenotype in HHT.

**Fgd1, the Cdc42GEF responsible for Faciogenital Dysplasia, directly interacts with cortactin and mAbp1 to alter osteoblast cell shape.** *J. Gorski, P. Hou, L. Estrada.* Dept Pediatrics & Human Genetics, Univ Michigan, Ann Arbor, MI.

FGD1 mutations result in Faciogenital Dysplasia (FGDY), an X-linked disease that affects skeletal formation and embryonic morphogenesis. FGD1 and Fgd1, the mouse FGD1 ortholog, encode guanine nucleotide exchange factors (GEF) that specifically activate Cdc42, a Rho GTPase that controls actin cytoskeletal organization. To further understand FGD1/Fgd1 signaling and begin to elucidate the molecular pathophysiology of FGDY, we demonstrate that Fgd1 directly interacts with cortactin and mouse actin-binding protein 1 (mAbp1), actin-binding proteins that regulate actin polymerization. In yeast two-hybrid studies, cortactin and mAbp1 Src homology 3 (SH3) domains interact with a single Fgd1 SH3-binding domain (SH3-BD). Biochemical far-western studies show that the Fgd1 SH3-BD directly binds to cortactin and mAbp1 *in vitro*; immunoprecipitation studies show that Fgd1 interacts with cortactin and mAbp1 *in vivo*. These analyses also show that Fgd1 SH3-BD mutations disrupt binding. Immunocytochemical analyses show that Fgd1 colocalizes with cortactin and mAbp1 in the subcortical actin cytoskeleton in cell ruffles, and that Fgd1 subcellular targeting is dynamic. Immunocytochemical studies also show that the cortactin SH3 domain targets Fgd1 protein to the subcortical actin cytoskeleton, and that abnormal Fgd1 targeting results in actin cytoskeletal abnormalities and marked changes in cell shape and viability. Furthermore, immunocytochemical analyses show that altered Fgd1/Cdc42 signaling alters osteoblast cell morphology. These data demonstrate that Fgd1 specifically and directly interacts with cortactin and mAbp1, and that these interactions play an important role in regulating cell shape. These data also indicate that Fgd1 plays a key role in regulating the actin cytoskeleton: by binding cortactin and mAbp1, Fgd1 is targeted to the dynamic cortical actin cytoskeleton; by activating Cdc42, Fgd1 activates NWASP to stimulate Arp2/3-directed actin polymerization. Thus, these data demonstrate that Fgd1/Cdc42 signaling plays a role in regulating the osteoblast actin cytoskeleton, and that Faciogenital Dysplasia is a disorder of FGD1/Cdc42 signaling.

**Gene expression profile of spinal muscular atrophy using microarrays.** *R. Olasso<sup>1</sup>, V. Joshi<sup>1</sup>, N. Roblot<sup>1</sup>, G.M. Lathrop<sup>2</sup>, M. Mayer<sup>3</sup>, J. Melki<sup>1</sup>.* 1) Molecular Neurogenetics Laboratory, INSERM, E.223, 2 rue Gaston Crmieux, 91057. Evry, France; 2) CNG, Evry; 3) Hopital Saint-Vincent de Paul, Paris.

Spinal Muscular Atrophy (SMA) is a common autosomal recessive neuromuscular disorder characterized by degeneration of motor neurons leading to progressive paralysis with muscular atrophy. SMA is caused by mutations of the Survival of Motor Neuron gene (SMN1). In addition, 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* 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. To characterize the degenerative process in SMA and identify the targets of SMN1 gene defect, comparative analysis of gene expression profiles have been performed using cDNA microarrays. Targeted tissues (skeletal muscle or spinal cord) of mutant mice (muscular or neuronal, respectively) were analyzed and compared to those of control mice at different stages of the disease course. Real time PCR of reverse transcripts was used to validate microarray results. Among the genes regulated, several protein families were shown to be involved including components of cytoskeleton, transcription factors and proteins implicated in proliferation/differentiation and metabolic pathways. A gene highly up-regulated in early stage of SMA disease progression in both types of mouse models has been selected for further investigation. Mice knock out for this candidate gene have been crossed to SMA mouse models to evaluate its role in SMA pathogenesis or disease progression. In addition, a first set of candidate genes has been selected to determine whether they are regulated in a similar manner in human SMA. To this end, real time PCR of reverse transcripts extracted from SMA tissue samples (skeletal muscle or spinal cord) or lymphocytes was performed. This study should help in designing biological markers for disease progression and in clarifying the SMA pathophysiology. Finally, they could also represent new potential targets for therapeutics in SMA.

**Functional absence of the tubulin-folding protein TBCE in the syndrome of hypoparathyroidism, mental and growth retardation, and facial dysmorphism.** *M.C. Huang<sup>1</sup>, M. Rubinstein<sup>2</sup>, B. Loeys<sup>3</sup>, R. Parvari<sup>2</sup>, G.A. Diaz<sup>1</sup>.* 1) Department of Human Genetics, Mount Sinai School of Medicine, New York, NY; 2) Ben Gurion University of the Negev, Beer Sheva, Israel; 3) Institute of Genetic Medicine, Johns Hopkins Hospital, Baltimore, MD.

Mutations in *TBCE* (tubulin-binding cofactor E) cause the syndrome of hypoparathyroidism, mental retardation, facial dysmorphism and growth failure (HRD or Sanjad-Sakati syndrome (SSS), MIM 241410; AR-KCS, MIM 244460). *TBCE* is a chaperone protein required for the proper folding of tubulin. Disruption of microtubule (MT) organization and organelle distribution has been described in disease cells homozygous for a deletion in a MT-binding domain (del52-55). We report studies in fibroblast cells from a compound heterozygous patient predicted to effectively express only a truncated protein, C371X. MT density was decreased in C371X cells relative to wildtype and del52-55 cells. However, MT-organizing centers had a more normal appearance than those in del52-55 cells. The distribution of the MT-dependent Golgi apparatus (GA) also showed mutation-specific differences. Staining in del52-55 mutant cells was diffuse and encircled the nucleus, while C371X cells displayed a more normal distribution. Differences between the mutants were also observed when tagged constructs were expressed in cultured cells. Despite disruption of the  $\alpha$ -tubulin binding domain, del52-55 sequestered  $\alpha$ -tubulin from MTs and tubulin monomers, leading to loss of cellular tubulin. In contrast, the C371X mutant did not destroy MTs despite accumulating to high levels in transfected cells, implicating the C-terminal end of the protein in  $\alpha$ -tubulin regulation. Immunoblotting with antibody to *TBCE* revealed that protein levels were greatly decreased in the mutant cell lines, suggesting that the mutants were unstable. These findings suggest that the disease phenotype results from near absence of cellular *TBCE*, with differences in the functional capacity of residual protein accounting for the divergent cellular phenotype. *TBCE* may be involved in MT dynamics or organization *in vivo*, rather than protein folding.

**Evidence for a DNA double-strand break-dependent activation of the Fanconi Anemia/BRCA pathway during the repair of DNA-interstrand crosslinks.** *A. Rothfuss, M. Grompe.* Molecular and Medical Genetics, Oregon Health&Sciences University, Portland, OR.

The detailed role of the FA/BRCA complex in the repair of DNA interstrand crosslinks (ICL) in human cells is not known. We therefore systematically investigated the involvement of the FA/BRCA complex in the initial steps of ICL repair in primary human fibroblasts. ICL repair in human cells consists of several steps, starting with a replication-independent recognition and incision of the lesion. The incised ICL are then processed further and DNA-double strand breaks (DSB) form as repair intermediates exclusively in S-phase, possibly when a replication fork becomes arrested at an incised ICL. We can show that Fanconi Anemia (FA) primary fibroblasts are fully proficient in these initial steps of ICL repair. The sensing and incision of ICL as well as the subsequent formation of DSB appeared normally in FA cells, suggesting a role of the FA/BRCA pathway downstream in ICL repair, after DSB have formed. Interestingly, analysis of the formation of monoubiquitinated FANCD2 indicates a slow activation of the FA/BRCA complex during ICL repair, which parallels the occurrence of DSB. In contrast, FANCD2 monoubiquitination occurs rapidly after ionizing radiation, consistent with the idea that the FA/BRCA complex is specifically activated upon DSB-formation. Using immunocytochemistry for the detection of FANCD2 foci, we can further show that this activation is restricted to a subpopulation of cells, which are also BrdU-positive. We therefore conclude that, while being dispensable for the early events in ICL repair, the FA/BRCA pathway is specifically activated in S-phase cells after DSB have formed as repair-intermediates. Most likely, this S-phase specific activation reflects a function of the FA/BRCA complex in the homologous recombination of DSB.

**Connexin 43 (Cx43) Mutations in Oculodentodigital Dysplasia (ODDD) Result in Gap Junction Dysfunction.**

*W.A. Paznekas<sup>1</sup>, A. Seki<sup>2</sup>, W. Coombs<sup>2</sup>, S.M. Taffet<sup>2</sup>, M. Delmar<sup>2</sup>, E.W. Jabs<sup>1</sup>.* 1) Johns Hopkins Univ, Balto, MD; 2) SUNY Upstate Medical Univ, Syracuse, NY.

ODDD, an autosomal dominant syndrome with high penetrance and phenotypic variability, has craniofacial and limb dysmorphisms. A thin nose with hypoplastic alae nasi and microphthalmia contribute to the distinctive facial appearance. Oral features include enamel hypoplasia, microdontia, and partial adontia, and digital anomalies consist of syndactyly in the feet and hands, involving digits 4 and 5 (Type III), camptodactyly, and clinodactyly. Spastic gait and progressive leukodystrophy are common. Conductive hearing loss, cataracts, glaucoma, keratoderma, and cardiac abnormalities can be observed. We found Cx43 gene mutations segregating in 49 affected members of 21 families. Cx43 codes for a protein that assembles into intercellular channels that regulate a variety of physiological and developmental processes through the exchange of small ions and signaling molecules. Mutations include: 18 amino acid substitutions (Y17S, S18P, G21R, G22E, K23T, A40V, Q49K, Q49P, R76S, L90V, V96A, Y98C, K102N, I130T, K134E, G138R, R202H, V216L) and two duplications (Q49dup, F52dup). The alterations occur throughout the protein except for the carboxyl terminus and third transmembrane region. Mutant and wildtype Cx43-GFP (Cx43 concatenated to GFP) were transiently transfected into N2A cells. Formation of gap junction plaques was assessed by fluorescence microscopy and the ability to form functional channels was evaluated by dual patch clamp. Three ODDD mutations localized in the 2nd half of the cytoplasmic loop of the Cx43 protein were found to affect channel electrical properties. Gap junction plaques were found on transfected cell pairs for all 3 mutants. I130T and K134E drastically diminished and mutation G138R eliminated formation of functional channels. Single channel analysis revealed a decrease in unitary conductance of K134E (54 pS) but no change for I130T (110 pS) when compared to wildtype (102 pS). These data suggest that the mutations significantly diminish the contribution of the affected allele in the formation of Cx43 channels in affected individuals.

**Identification of additional transcripts in the facioscapulohumeral muscular dystrophy region on human chromosome 4q35.** *J. Clapp, D.J. Bolland, A. Jenkinson, J.E. Hewitt.* Institute of Genetics, University of Nottingham, Nottingham, UK.

Facioscapulohumeral muscular dystrophy (FSHD) is caused by a unique genetic rearrangement close to the telomere of human chromosome 4q. The disease is causally associated with deletions within a tandem DNA repeat (D4Z4). A position effect mechanism has been proposed whereby loss of D4Z4 subunits leads to upregulation of genes in the 4q35 region. A recent study has indicated that mRNA levels of three genes (*FRG1*, *FRG2* and *ANTI*) may be increased in FSHD patient muscle. However, the physical distance encompassing these genes is almost 5Mb and is poorly characterized at the genomic level, thus there may be other genes whose expression is perturbed by the FSHD mutation. We have used a combination of bioinformatics, Northern blotting and RT-PCR to produce a detailed analysis of the gene complement and expression profiles for the distal 5.5Mb of 4q. Thus far, 29 transcripts have been identified, many of which are expressed in fetal or adult skeletal muscle and are currently being examined as candidates for a role in FSHD. The majority of the genes are clustered in the proximal 2.5Mb of 4q35. We have examined in detail the 3Mb interval between the cadherin gene *FAT* and *FRG1* at the distal end of this region as this has been especially poorly characterized. Interestingly, we have found at least two transcripts in this interval that appear to represent spliced, non-coding RNAs. To investigate the evolutionary history of the FSHD region and to help gene identification strategies, we have carried out comparative mapping studies in mouse and puffer fish. In the homologous region on mouse chromosome 8, gene content, distribution and density are similar to human. However, *FRG2* and D4Z4 are not conserved and the *Fat-Frg1* interval is some 0.5Mb larger than the human equivalent region. Although homology of synteny is less extensive in *Takifugu* and *Tetraodon*, the *FAT-FRG1* linkage group is conserved in both these genomes with the two genes separated by 100-120kb in both puffer fish, suggesting there may be important sequences in this interval.

**Dominant lamin A/C gene mutations can be associated to neuropathy.** *S. Benedetti<sup>1</sup>, S. Previtali<sup>1</sup>, D. Toniolo<sup>1</sup>, S. Iannaccone<sup>1</sup>, A. Quattrini<sup>1</sup>, P. Carrera<sup>1</sup>, M. Ferrari<sup>1</sup>, E. Bertini<sup>2</sup>.* 1) D.R. San Raffaele, IRCCS, Milano, Italy; 2) Bambino Gesù, IRCCS, Roma, Italy.

The coexistence of neurogenic and myogenic etiology in scapuloperoneal syndrome was not previously ascribed to a single gene. Defects in the nuclear envelope protein lamin A/C, initially described in Emery-Dreifuss muscular dystrophy, have been associated to a variety of pathologies, affecting mainly muscular and adipose tissues. Recently, mutations in the lamin A/C encoding gene (LMNA) have been reported in families affected by autosomal recessive Charcot-Marie-Tooth type 2 neuropathy. We describe here two patients associating features of myopathy and neuropathy linked to dominant LMNA mutations. Patient 1 presented with tip walking, lower limb weakness and moderately elevated serum CK. Neurophysiological studies revealed a sensory-motor axonal neuropathy, whereas muscle biopsy showed signs of both neurogenic and myogenic features. Molecular analysis of LMNA gene revealed a heterozygous R571C substitution in a conserved region of the lamin C specific C-terminal domain. Interestingly, another mutation affecting the same codon (R571S) has been previously described and associated to a completely different phenotype, characterized by atrial fibrillation and dilated cardiomyopathy with no signs of skeletal muscle involvement. Patient 2 presented mild proximal weakness and fatigability. She was carrying a pacemaker for cardiac arrhythmia and had a familiar history of sudden death. Needle electromyography showed mild myopathic changes. A biopsy specimen from the quadriceps revealed chronic myogenic and neurogenic features including atrophic angular fibers and type grouping. Analysis of the LMNA gene showed a heterozygous four nucleotides deletion in exon 5 (864del4), leading to premature protein truncation. The same mutation was found in the asymptomatic younger brother. This report describes for the first time dominantly-inherited alterations of the nuclear lamins affecting both muscle and peripheral nerve. It also underlines the intra- and inter-familial clinical heterogeneity of LMNA mutations, suggesting the involvement of modifier genes in determining the phenotype.

**A West European cluster of severe cardiac and skeletal myopathy associated with a de novo R406W mutation in desmin.** A. Dagnadorj<sup>1</sup>, M. Olivé<sup>2</sup>, J-A. Urtizberea<sup>3</sup>, M. Halle<sup>4</sup>, A. Shatunov<sup>1</sup>, C. Bönnemann<sup>5</sup>, K-Y. Park<sup>1</sup>, H. Goebel<sup>6</sup>, I. Ferrer<sup>2</sup>, P. Vicart<sup>7</sup>, M. Dalakas<sup>1</sup>, L. Goldfarb<sup>1</sup>. 1) National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, US; 2) Institut de Neuropatologia, Ciutat Sanitària i Universitària de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain; 3) Hopital Raymond Poincaré, Garches, France; 4) Medizinische Klinik, Georg-August-Universität, Goettingen, Germany; 5) Division of Neurology, The Children's Hospital of Philadelphia, Philadelphia, PA, US; 6) Department of Neuropathology, Johannes Gutenberg-University Medical Center, Mainz, Germany; 7) Laboratoire Cytosquelette et Développement, Faculté de Médecine Pitié-Salpêtrière, Paris, France.

This study has identified four patients originating from Greece, Spain and Germany who suffered from progressive cardiac arrhythmia and conduction blocks followed by muscle weakness and atrophy in the lower extremities subsequently spreading to the upper limbs, trunk and neck muscles. Each patient needed a permanent pacemaker or an ICD, all four became severely incapacitated in their twenties-early thirties, and one died of cardiac complications at the age of 28 years. Skeletal muscle biopsies showed intracytoplasmic aggregates strongly reacting with anti-desmin antibodies. A R406W mutation was identified in each patient. The pathogenic potential of the desmin R406W mutation was documented by its deleterious effects in the SW13 (vim-) cells. The mutation was not present in patients parents, although paternity check did not identify alleles coming from a different source but the parents. The mutation-carrying chromosomes showed no similarity, clearly indicating that the R406W substitution is a recurrent de novo mutation. Structural analysis of mutant desmin protein performed by ESyPred3D automated homology modeling indicates that the high pathogenic potential of this mutation results from disabling of the highly conserved YRKLLEGEE motif at the C-terminal end of the 2B helix that has a critical role in desmin filament assembly.

**Phenotypic and molecular characterization of the *myd* mouse, a model of glycosylation-deficient muscular dystrophies.** C.J. Moore<sup>1</sup>, P.K. Grewal<sup>1</sup>, R. Herbst<sup>2</sup>, T. Dechat<sup>3</sup>, R. Bittner<sup>3</sup>, J.E. Hewitt<sup>1</sup>. 1) Institute of Genetics, University of Nottingham, Nottingham, UK; 2) Brain Research Institute, University of Vienna Medical School, Austria; 3) Neuromuscular Research Department, Institute of Anatomy, University of Vienna, Austria.

Within the last two years mutations in four genes encoding glycosylation enzymes have been shown to cause muscular dystrophy; *POMGnT1* (Muscle Eye Brain disease), *POMT1* (Walker Warburg syndrome), *Fukutin* (Fukuyama congenital muscular dystrophy) and *FKRP* (congenital muscular dystrophy type 1C and Limb girdle muscular dystrophy 2I). *POMT1* and *POMGnT1* are both involved in *O*-mannosyl glycosylation, the activities of the other two proteins are not known. Abnormal glycosylation is thus implicated as an important mechanism underlying a subset of muscular dystrophies. Aberrant glycosylation of the dystrophin-associated protein -dystroglycan (-DG) is present in all these diseases, suggesting these genes act in a common pathway. Recently, we showed that the causative mutation in the myodystrophy (*myd*) mouse is also in a putative glycosyltransferase gene, *Large*. The *myd* mouse has progressive muscular dystrophy, neuronal migration defects and a mild cardiomyopathy, associated with hypoglycosylation of -DG in brain and muscle. Now, we show that the neuromuscular junctions in *myd* mice are irregularly distributed. Staining for synaptophysin shows aberrant and irregular sprouting of nerve terminals at the synaptic region. To investigate specific glycosylation changes in *myd* muscle we have used lectin overlay and pull-down assays. Several lectins that display differential binding to -DG from *myd* and wild type muscle have been identified. An unusual feature of the *Large* protein is the presence of a coiled-coil region, which may be involved in protein-protein interactions. Therefore, we have used a Sos recruitment based, yeast-two-hybrid screen using *Large* as bait and identified six potential interactors. Our study of the *myd* mouse may help us to dissect the molecular pathways underling glycosylation-deficient muscular dystrophies.

**Allelic heterogeneity of SMARD1 at the IGHMBP2 locus : identification of nine novel mutations.** *I. Maystadt*<sup>1,2,3</sup>, *M. Zarhrate*<sup>1</sup>, *C. Verellen*<sup>2</sup>, *A. Munnich*<sup>1</sup>, *L. Viollet*<sup>1</sup>. 1) INSERM U393, Necker Hospital, Paris, France; 2) Department of Human Genetics, Catholic University of Louvain, Brussel, Belgium; 3) FNRS, Brussel, Belgium.

Spinal Muscular Atrophy with Respiratory Distress (SMARD), also known as Diaphragmatic Spinal Muscular Atrophy or Distal Hereditary Motor Neuropathy type VI (dHMNVI), is an autosomal recessive disorder characterized by neurogenic muscular atrophy due to progressive anterior horn cell degeneration, and by early life-threatening respiratory failure due to diaphragmatic dysfunction. Spinal muscular atrophy with respiratory distress is clinically and genetically heterogeneous. SMARD type 1, characterized by the onset of respiratory failure within the first weeks of life, has been ascribed to chromosome 11q13-q21 (Grohmann et al, 1999). In six SMARD1 families, seven mutations were identified in the IGHMBP2 gene, encoding the immunoglobulin mu-binding protein 2 (Grohmann et al, 2001). This gene 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 the identification of nine novel IGHMBP2 mutations, in five patients with a typical SMARD1 phenotype, beginning at the age of one to six months. Eight of these mutations were detected by DHPLC analysis of the IGHMBP2 coding sequence and all were confirmed by direct double strand DNA sequencing. Seven mutations were missense (Q196R, P216L, L251P, D565B, L577P, R603C, R637C), and two were nonsense mutations (C496X, R790X). None of them were found in at least 100 control chromosomes from a group of healthy individuals. Except for the nonsense mutation R790X in exon 13, all these novel mutations occurred in the putative DNA helicase domain of the gene, as for mutations previously reported. The mutations occurred at highly conserved residues, strongly supporting their pathogenic nature. The identification of novel IGHMBP2 variants will help to diagnosing SMARD1 and may contribute to the functional characterization of IGHMBP2 gene product.

**Muscleblind is Associated with Splicing Alterations in Myotonic Dystrophy.** *L.E. Machuca-Tzili, M. Fardaei, H. Thorpe, J.D. Brook.* Institute of Genetics, University of Nottingham, Nottingham, United Kingdom.

Myotonic dystrophy (DM1) is the most frequent autosomal dominant myopathy in adults. The cardinal features of DM are myotonia, muscle wasting and weakness, cataract, hypogonadism, frontal balding and ECG changes. The mutation underlying DM1 is an unstable triple repeat (CTG) located in the 3-UTR of the DMPK gene. Recently a second form of the disease (DM2) has been identified which is caused by an expansion of the CCTG tetranucleotide repeat in a different gene, ZNF9.

Several models have been proposed to explain the molecular basis of myotonic dystrophy. The RNA dominant hypothesis proposes that an expanded CUG or CCUG-containing transcript could sequester specific RNA binding proteins leading to aberrant alternative splicing. One of these proteins is Muscleblind, which is encoded for three different genes (MBNL1, MBNL2, MBNL3). The expanded transcripts accumulate as RNA nuclear foci in DM1 and DM2 cells and co-localise with MBNL proteins. There is evidence of dysregulation of splicing of different genes in DM1 patients.

In order to test if the Z-band disruption in DM is related to alterations in the alternative splicing of Z-band transcripts, RT-PCR studies were carried out in muscle biopsies from DM patients looking at the alternative isoforms of the sarcomeric gene ZASP. At the same time gene knockdown studies were performed in mouse myoblasts (C2C12) to elucidate the role of MBNL proteins in these events. Specifically RNAi was used to knock-down the three Mbnl genes. There is significant difference in the splicing pattern of ZASP transcripts between normal and DM human samples, and in the mouse homolog between normal and knock-down cells. Our results suggest that ZASP represents a novel target for abnormal mRNA splicing in Myotonic Dystrophy.

**Gene expression profiling of Fukuyama-type congenital muscular dystrophy -Neurogenic terminal maturational delay in muscle fibers.** *M. Taniguchi<sup>1</sup>, H. Kurahashi<sup>1,2</sup>, S. Noguchi<sup>3</sup>, J. Sese<sup>4</sup>, T. Tsukahara<sup>3</sup>, I. Nishino<sup>3</sup>, S.*

*Morishita<sup>4</sup>, T. Toda<sup>1</sup>.* 1) Div Functional Genomics, Osaka Univ Grad Sch Med, Osaka; 2) Div Mol Genetics, Fujita Health Univ, Aichi; 3) Dept Neuromuscul Res, NCNP, Tokyo; 4) Dept Computational Biol, Tokyo Univ Grad Sch Frontier Sci, Tokyo, Japan.

Fukuyama-type congenital muscular dystrophy (FCMD), Walker-Warburg syndrome, and muscle-eye-brain (MEB) disease are similar autosomal recessive disorders characterized by CMD, lissencephaly due to a defect during neuronal migration, and eye anomalies. We have identified the genes for FCMD and MEB, which encode the fukutin protein and the POMGnT1 glycosyltransferase. These are thought to modulate the glycosylation of  $\alpha$ -dystroglycan, and mutations in such glycosyltransferases are known to result in posttranslational disruption of dystroglycan-ligand interactions in skeletal muscle and brain in these congenital muscular dystrophies. To investigate the pathophysiology of this  $\alpha$ -dystroglycanopathy, we analyzed gene expression profiling of skeletal muscle in FCMD by using cDNA microarray. In FCMD muscle, extracellular components were highly expressed through all stages of the disease irrelevant to the severity of fibrosis. Gene expression of muscle components were not highly upregulated compared to other types of muscular dystrophy. Slow type myosin heavy chain was dramatically downregulated. Histochemical analysis showed the increased rate of type 2C fibers in FCMD. These imply that FCMD muscle is in active fibrotic phase whereas the muscle fibers are in immature state. Real-time quantitative PCR confirmed that agrin, a key protein in postsynaptic muscle differentiation through neuromuscular junction, was highly upregulated in FCMD muscle; the downstream genes such as Musk and synaptophysin were in low expression. These suggest that the main pathophysiology of FCMD might be the disorder of terminal muscle fiber differentiation induced by the disruption of neuromuscular signal transmission. Though severe necrotic degeneration or wasting of muscle fibers has been thought as the main result of the fibrotic change in CMD, differentiatinal change of immature muscle fibers may lie in the pathophysiologic change of FCMD.

**Detection of thirty novel mutations in the *FBNI* gene in patients with Marfan syndrome or related fibrillinopathy. The Australian perspective.** L.C. Ades<sup>1,2,3</sup>, K.J. Holman<sup>1,4</sup>, M. Brett<sup>1,4</sup>, B. Bennetts<sup>4</sup>, A. Biggin<sup>1</sup>. 1) Marfan Research Group; 2) Department of Clinical Genetics; 3) Discipline of Paediatrics and Child Health; 4) Department of Molecular Genetics. The Children's Hospital at Westmead, Westmead, NSW, Australia.

Marfan syndrome (MFS) is a disorder of the extracellular matrix caused by mutations in the gene encoding fibrillin-1 (*FBNI*). Recent studies have illustrated the variability in disease severity and clinical manifestations of MFS. Clear evidence for useful genotype-phenotype correlations has been slow to emerge.

We have screened 57 unrelated patients with MFS or a Marfan-like phenotype using a combination of single strand conformation polymorphism (SSCP) and/or denaturing high performance liquid chromatography (DHPLC). We were able to detect 49 different *FBNI* mutations, 30 (62%) of which were novel. The mutations comprised 37 substitutions (76%), 10 deletions (20%), 1 duplication (2%) and 1 insertion-deletion (2%). There were 29 missense (59%), 9 frameshift (18%), 8 splice site (16%) and 3 nonsense mutations (6%).

A custom database containing detailed clinical information and results from *FBNI* mutation screening was developed in order to analyse possible genotype-phenotype correlations. Patients with an identified *FBNI* mutation were more likely to have ectopia lentis (EL) and cardiovascular complications compared to those without an identifiable mutation (p0.05, relative risks of 4.7 and 2.0, respectively). EL was also found to be more prevalent in patients whose mutations involved cysteine residues (p0.05, relative risk 1.6). The data presented here demonstrate an overall mutation detection of 86%. This increased to a detection of 93% when considering only DHPLC analysis of patients meeting the Ghent criteria.

DHPLC is a reliable and robust technique for *FBNI* mutation detection. We plan to continue to collate detailed clinical information and mutation screening data with the aim of elucidating further genotype-phenotype correlations.

**Factor IX mutations in Korean hemophilia B patients.** *Y.H. Cho, H.K. Seo, J.H. Kyhm, E.S. Shin, C.H. Lee.*  
Department of Medical Genetics, College of Medicine, Hanyang Univ, Seoul 133-791, Korea.

Hemophilia B or Christmas disease is an X-linked recessive bleeding disorder due to mutations of the coagulation factor IX gene. Hemophilia B is known to be caused by a variety of mutations, which can be found in the whole coding regions. The purpose of this study was to find out molecular defects of factor IX gene in Korean hemophilia B patients, and to establish a method for prenatal and carrier diagnosis. The promoter region, eight exons and their intron boundaries were amplified using PCR in 26 DNA samples. The PCR products were subcloned into pGEM-T easy plasmid vector and transformed into JM109 high efficiency competent cells. DNA sequencing of each PCR product was performed by cycle sequencing with ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kits(Ver. 2.0) and ABI PRISM 310 automated sequencer. With direct sequencing analysis, mutations were detected in 23 cases. Ten cases were found to harbor point mutations in exon H, 3 cases each in exon B and G, 2 cases in exon E, and one case each in exon C, D, and E respectively. Two cases of splice site mutations were found at intron 4 and 7. In the sequence analysis, 13 cases were transition, and 9 cases were transversion. Thirteen cases were missense mutations, 7 cases were nonsense mutations, one case was frameshift mutation due to single base deletion, and 2 cases were splice site mutations. Fifteen mutations were known by previous studies, and 8 cases were novel mutations found in this study. This study revealed that the mutation pattern in factor IX gene of Korean hemophilia B patients is extremely heterogeneous and wide spread over whole coding regions, although majority of mutations were found at exon H. And the direct mutation analysis will be very useful for the prenatal and carrier diagnosis as complementary to indirect linkage analysis.

**Eight novel mutations revealed by DHPLC analysis of the neurofibromatosis type 1 (NF1) gene in southern Italian NF1 patients.** *A. Gabriele<sup>1</sup>, M. Ruggieri<sup>2,3</sup>, G. Peluso<sup>1</sup>, T. Sprovieri<sup>1</sup>, A. Patitucci<sup>1</sup>, A. Magariello<sup>1</sup>, R. Mazzei<sup>1</sup>, F.L. Conforti<sup>1</sup>, S. Genovese<sup>2</sup>, E. Ciancio<sup>2</sup>, A. Gambardella<sup>4</sup>, M. Muglia<sup>1</sup>, Molecular genetics.* 1) ISN, CNR, Cosenza, Italy; 2) ISN, CNR, Catania, Italy; 3) DEPARTMENT OF PAEDIATRICS, UNIVERSITY Catania, Italy; 4) INSTITUTE OF NEUROLOGY, UNIVERSITY OF MAGNA GRAECIA, Catanzaro, Italy.

Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder with a prevalence of 1 in 3500 individuals in the general population and is characterized by pigmentary changes, neurofibromas and systemic complications. We performed, by means of denaturing high performance liquid chromatography (DHPLC) and DNA sequencing, molecular analysis in 27 exons of the NF1 gene in a panel of 85 consecutive NF1 patients from southern Italy. DHPLC is largely automated heteroduplex-based technique optimised, in this study, for the rapid screening of mutations in exons 4a, 4b, 4c, 5, 10a, 10b, 10c, 14, 15, 16, 19a, 19b, 20, 21, 22, 23.1, 23.2, 23a, 24, 25, 26, 27a, 27b, 29, 34, 37 and 41 of the NF1 gene. Mutational screening of the NF1 gene revealed the presence of sixteen different mutations. In particular, eight represented known mutations: 165 del TGTT, R440X, Y489C, H781P, 1152 del ACTC, R1276X, K1423E, 2162 ins.A and eight were novel mutations. Of the eight novel mutations two created a stop codon (E1192X, Q1360X), two were nucleotide substitutions (L1109F, N1394D), two were small insertions (1504 insA, 2265 insC), one was a small deletion (1503delG), and another was a splice site mutation (IVS24+1GA). None of the novel mutations was detected in 100 control chromosomes. In conclusion, we have confirmed the possibility for routine molecular diagnosis in NF1 by direct mutation detection using DHPLC that appears to be a simple, rapid and efficient methodology. The identification of these eight novel mutations contributes to the definition of the NF1 gene germ-line mutational spectrum.

**Novel single base pair deletion of the CYP1B1 gene in a family with primary congenital glaucoma.** *S. Cuevas Sr<sup>1</sup>, C. Chima<sup>1</sup>, L.M. Gonzalez<sup>1</sup>, M.R. Rivera<sup>1</sup>, O. Messina<sup>2</sup>, I. Babayan<sup>2</sup>.* 1) Genetica, Hospital General de Mexico, Mexico DF, Mexico; 2) Oftalmologia, Hospital General de Mexico, Mexico DF, Mexico.

Primary congenital glaucoma (PCG) is an uncommon disease with an incidence that ranges from 1 in 1,1250 to 1 in 22,000 births in several populations. Although some PCG patients are sporadic cases, PCG appears to harbor autosomal recessive mode of inheritance. PCG has been linked to homozygous mutations in the CYP1B1 gene that is located on chromosome 2p22-p21. The CYP1B1 gene contains 3 exons with an ORF of 1,629 bp starting in the second exon, and harbors nine TCDD-responsive (dioxin-responsive) enhancer core binding motifs. The CYP1B1 gene codifies for cytochrome P4501B1, a member of a subfamily of cytochrome P450. Mutations and polymorphisms have been identified in the CYP1B1 gene. Mutations affecting the conserved core structures of cytochrome P4501B1 result in PCG while mutations in other regions hold the potential to define differences in the estrogen metabolism. In the present study, we describe a family with PCG due to a novel single base pair deletion within codon 370 (C1453del) of the CYP1B1 gene. This family also harbored a novel polymorphic variant of the cytochrome P4501B1 with six single nucleotide polymorphisms (SNPs) not previously reported.

**Mutation screening in patients with hereditary haemorrhagic telangiectasia.** *P. Bayrak Toydemir, J. McDonald, K. Ward, R. Mao.* DNA Diagnostic Lab, University of Utah, Salt Lake City, UT.

Hereditary Haemorrhagic Telangiectasia (HHT) is an autosomal dominant disorder characterized by vascular dysplasia leading to telangiectases and recurrent hemorrhage. The disease exhibits variability in clinical expression and age of onset. Mutations in at least two genes, endoglin (*ENG*) on chromosome 9 region q34 and activin A receptor type II-like 1 (*ALK1* or *ACVRL1*) on chromosome 12 region q13 have been shown to be associated with the disease.

We developed a cascade mutation screening assay for *ENG* and *ALK1* genes and analyzed a total of 22 unrelated families diagnosed by HHT. Conformation sensitive gel electrophoresis (CSGE) was used to screen mutations in the coding regions of both genes. The variants seen in CSGE were confirmed by a follow up sequencing analysis in both directions. Subsequently, the samples negative in the CSGE will be sequenced to completion for the whole coding regions of *ENG* and *ALK1*.

By using linkage data, when available, and our screening assay we have found *ENG* mutations in 6 families and *ALK1* mutations in 4 families. In 2 families disease causing mutations were not detected by CSGE or sequencing although linkage was shown to *ENG* locus in one family and to *ALK1* locus in the other. In these cases, presence of large deletions could not be excluded with our method. One family did not show linkage to either *ENG* or *ALK1* loci, suggesting a third locus.

To date we have found mutations in 10 of 22 HHT patients. We are continuing to analyze the remaining patients. The identification of the mutations in HHT patients is helpful for surveillance of the affected individuals and family members in risk.

**Screening for Sequence Variations in the von Willebrand Factor Gene using DHPLC.** *D.B. Bellissimo<sup>1</sup>, J.K. Kakela<sup>1</sup>, K.D. Friedman<sup>1</sup>, J.C. Gill<sup>2</sup>, R.R. Montgomery<sup>2</sup>.* 1) Blood Center of Southeastern WI, Milwaukee, WI; 2) Medical College of Wisconsin, Milwaukee, WI.

Von Willebrand disease (vWD) is a common inherited bleeding disorder caused by quantitative (types 1 and 3) and qualitative (type 2) defects in von Willebrand Factor (vWF). Many of the type 2 mutations are localized to exons 18-20 (type 2N) and exon 28 (types 2A, 2B and 2M) making them amenable to DNA sequence analysis. However, the analysis of vWF mutations outside these localized exons is complicated by the large size of the vWF gene that is ~180 kb, containing 52 exons and whose mRNA is 8.7 kb. A simple and sensitive method to scan genes for potential mutations would be useful for vWD and other genetic disorders. The goal of this study was to determine the sensitivity and feasibility of the denaturing high performance liquid chromatography (DHPLC) system for detection of mutations and polymorphisms in the vWF gene. Primers were designed to amplify each exon while avoiding amplification of the vWF pseudogene. Exon-specific primers were tagged with sequencing primers, allowing direct sequencing of each vWF exon. The primers were designed with GC-clamps in order to optimize the melt profile of each vWF amplicon. All sequence variations in 33 previously characterized vWD samples were detected using DHPLC, making the technique one hundred percent sensitive in comparison to DNA sequencing. The sequence variations were distributed among various vWF exons including exons 4, 12, 17, 18, 19, 20, 28, 37 and 52 as well as representing vWD types 1, 2A, 2B, 2M and 2N. In addition, we identified 16 novel potential mutations or polymorphisms in 15 vWD patients. Four of the mutations have clear functional consequences. Two nonsense mutations were identified in exons 3 (R34X) and 28 (R1566X). A deletion in exon 5 (~124 bp) and a deletion in exon 23 (~8 bp) were also found. The functional aspects of the remaining 12 sequence variations need to be assessed. Six of the eight variations within exons changed the amino acid. Four variations in introns were also identified. We conclude that DHPLC is a useful tool for scanning the vWF gene and identifying sequence variations.

**Use of Temperature Gradient Capillary Electrophoresis (TGCE) for Complete Scanning of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene.** *L. Chou*<sup>1</sup>, *E. Lyon*<sup>1,2</sup>. 1) Research and Development, ARUP Laboratories, Salt Lake City, UT; 2) Pathology Department, University of Utah.

Mutation analysis reveals DNA variation associated with complex inherited diseases. Many of these diseases involve large genes with many different mutations. Direct sequencing identifies these mutations but is laborious and time consuming in both set-up and analysis. Emerging technologies to scan large genes for any heterozygous alteration include denaturing high performance liquid chromatography (D-HPLC) and temperature gradient capillary electrophoresis (TGCE). These technologies detect heteroduplexes that form from re-annealed heterozygous samples. Any alteration detected is confirmed by sequence analysis for the specific region of the gene. This study focused on scanning for mutations in the cystic fibrosis transmembrane regulator (CFTR) gene using TGCE. We designed exon-specific primers to amplify the entire coding region of the CFTR gene using standard PCR conditions. An initial sample set that included 20 mutations covering 8 exons and 4 introns were identified by oligo ligation assay (OLA). TGCE scanning showed 100% agreement with OLA for heterozygous samples. Since homozygous mutants do not form heteroduplexes, these samples require mixing with a known wild type allele to form artificial heteroduplexes. De-identified clinical samples with no mutation detected by OLA were subsequently scanned for mutations in all 27 exons. Results were confirmed by bi-directional sequence analysis, showing 100% concordance. Among 12 randomly chosen samples with no identified mutations by OLA, only 2 samples (17%) were found to be true wild types in all 27 exons scanned. Ten samples had known polymorphisms. One sample had a previously reported mutation in intron 14b (2789+2insA), a possible mRNA splicing defect. We also found a mutation at exon 20 (3991 G/A), which changed the amino acid from glycine to arginine (G1287R) and is not reported in the current database. In conclusion, TGCE has a great potential for use in a high throughput clinical environment for mutation scanning, thus reducing sequencing cost and effort.

**Germline fumarate hydratase mutations and evidence for a founder mutation underlying multiple cutaneous and uterine leiomyomata.** *G.S. Chuang<sup>1</sup>, A. Martinez-Mir<sup>1</sup>, L. Horev<sup>2</sup>, B. Glaser<sup>2</sup>, A. Geyer<sup>1</sup>, M. Landau<sup>3</sup>, A. Waldman<sup>3</sup>, D. Gordon<sup>1</sup>, L.J. Spelman<sup>4</sup>, I. Hatzibougias<sup>5</sup>, D.E. Engler<sup>1</sup>, P.B. Cserhalmi-Friedman<sup>1</sup>, J. Green<sup>6</sup>, M.P. Garcia Muret<sup>7</sup>, M. Prieto Cid<sup>7</sup>, S. Brenner<sup>8</sup>, E. Sprecher<sup>9</sup>, A.M. Christiano<sup>1</sup>, A. Zlotogorski<sup>2</sup>.* 1) Departments of Dermatology and Genetics & Development, Columbia University, New York, NY; 2) Department of Dermatology, Hadassah Medical Center, Jerusalem, Israel; 3) Departments of Dermatology, Wolfson Medical Center, Holon, and Haemek Medical Center, Afula, Israel; 4) South East Dermatology, Brisbane, Queensland, Australia; 5) Institute of Pathology, Thessaloniki, Greece; 6) Department of Dermatology, St. Vincent's Hospital, Melbourne, Australia; 7) Servicio de Dermatología, Hospital de Sant Pau, Barcelona, Spain; 8) Department of Dermatology, Sourasky Medical Center, Tel-Aviv, Israel; 9) Department of Dermatology, Rambam Medical Center, Haifa, Israel.

Multiple cutaneous and uterine leiomyomata syndrome (MCL) is an autosomal dominant disease characterized by the presence of benign tumors of smooth muscle origin (leiomyoma) in skin and uterus in female, and skin leiomyoma in male patients. MCL can also be associated with type II papillary renal cell cancer (HLRCC). The locus for MCL and HLRCC was recently mapped to chromosome 1q42.3-q43 and subsequently, dominantly inherited mutations in the fumarate hydratase gene (FH) were identified. Here we report the clinical and genetic analysis of 16 families with MCL. Mutational analysis of the FH gene revealed a total of 13 different mutations accounting for the disease in all families. We have identified missense, nonsense, splicing, in-frame deletions and frameshift mutations. All of them are unique to single families, with the exception of the splicing mutation 905-1G>A, identified in four families of Iranian origin. The analysis of highly polymorphic microsatellite markers in the vicinity of the FH gene showed a shared haplotype in these four families, suggesting that 905-1G>A represents a founder mutation. Collectively, we have identified 10 novel and three previously described FH mutations that further support the role of FH in the pathogenesis of MCL.

**Two novel mutations in the *PRX* gene in patients with demyelinating Charcot-Marie-Tooth disease: evidence for intrafamilial phenotypic variability.** *M. Cesani, M. Milani, S. Baratta, C. Caccia, D. Pareyson, F. Taroni.* Div. Biochemistry & Genetics, Ist. Naz. Neurologico "C. Besta", Milan, Italy.

Hereditary peripheral neuropathies are a clinically and genetically heterogeneous group of disorders that can be inherited as autosomal dominant (AD), autosomal recessive (AR) or X-linked traits. The sensory and motor demyelinating forms represent the most common inherited peripheral nerve diseases and constitute a wide phenotypic spectrum which includes Charcot-Marie-Tooth disease type 1 (CMT1), Dejerine-Sottas disease (DSD), congenital hypomyelinating neuropathy, and hereditary neuropathy with liability to pressure palsies. At least 20 genetic loci and twelve genes have been associated with the demyelinating form of CMT and related disorders. A limited number of loci have been linked to the rare autosomal recessive forms of CMT1. Mutations in the gene coding for periaxin (*PRX*), a cytoskeleton-associated protein expressed in the noncompact peripheral myelin, have been shown to cause both AR-CMT (CMT4F) and DSD in a small number of patients. Using DHPLC and sequence analysis, we have screened for *PRX* mutations a group of 30 patients with severe demyelinating neuropathy, negative for the common 17p11 duplication and mutations in the *MPZ*, *PMP22* and *GJB1* genes. In one family, two siblings with a severe form of demyelinating neuropathy were found to be compound heterozygotes for two novel *PRX* mutations, one frameshift mutation (2688delC) and one nonsense mutation (1908C->T). Both mutations were located in exon 7, affecting only the long variant of the protein (L-periaxin). Interestingly, the two patients exhibited a different phenotype. One sib had an early-onset very severe DSD phenotype characterized by motor delay and profound reduction in motor conduction velocities (<6 m/sec) while the other had a milder phenotype with later onset and motor conduction velocities of 10-15 m/sec. Nerve biopsy was performed in the first patient showing severe fiber loss and onion-bulb formation. (Partially supported by a grant Ricerca Finalizzata Ministero della Sanità to F.T.).

**Screening candidate genes for mutations using genome custom resequencing microarrays.** *P. Chudgar*<sup>1</sup>, *N. Xu*<sup>1</sup>, *L.P. Chorich*<sup>1</sup>, *C. Liu*<sup>2</sup>, *J.A. Warrington*<sup>2</sup>, *L.C. Layman*<sup>1,3</sup>. 1) Dept. OB/GYN, Medical Coll GA, Augusta, GA; 2) Affymetrix, Inc., Santa Clara, CA; 3) Neurodevelopmental Biology Program, Inst Mol Med Genet, Med Coll GA, Augusta, GA.

Patients with idiopathic hypogonadotropic hypogonadism (IHH) present with absent puberty, infertility, and low gonadotropins. To date, mutation analysis of these genes has been performed utilizing traditional PCR-based techniques, such as SSCP or DGGE, followed by DNA sequencing. The use of genome custom resequencing microarrays allows a high throughput technology to detect gene mutations, whereby 30kb of sequence from multiple genes can be screened per patient per chip. However, because this is a relatively new application of this technology there exists some uncertainty about its efficacy for mutation detection in research laboratories. In this pilot study we screened 8 IHH patients for mutations in six different genes using microarrays. Long range PCR was performed and PCR products for each patient sample were pooled, purified, and fragmented. PCR products were then labeled with biotin, hybridized individually to a microarray, washed, stained with streptavidin, scanned, and analyzed by the ABACUS program. For six chips, 92-93% of bases were called with high confidence scores, while for two patients only 67% of bases were called. A total of 38 single nucleotide polymorphisms (SNPs) were identified from 30kb in these eight patients, including 12 in exons, 9 in the promoter/5 untranslated region, 5 in 3 untranslated region, 9 in introns, and 3 that are being determined. To date, 18 of 19 (94.7%) SNPs have been confirmed by DNA sequencing, while the others are currently being sequenced. Since only 67% of bases were called correctly on two chips, the presence of genomic rearrangement is currently being evaluated. Twenty-four additional arrays are currently being completed. The use of custom resequencing microarrays has already permitted the identification of new gene mutations in IHH patients. The results of these studies will determine the feasibility of using custom resequencing microarrays for mutation detection in disease studies carried out in individual research laboratories.

**Genomic structure and novel mutations in the *TRIM37* gene in Mulibrey nanism.** *R. Hämäläinen<sup>1</sup>, K. Avela<sup>1</sup>, J. Kallijärvi<sup>1</sup>, M. Lipsanen-Nyman<sup>2</sup>, A.-E. Lehesjoki<sup>1</sup>.* 1) Folkhälsan Institute of Genetics and Department of Medical Genetics, Biomedicum Helsinki, University of Helsinki, Finland; 2) The Hospital for Children and Adolescents, and Helsinki University Central Hospital, University of Helsinki, Finland.

Mulibrey nanism is an autosomal recessive growth disorder of unknown pathogenesis. It belongs to the diseases of the Finnish disease heritage and 80 % of the approximately 100 patients diagnosed worldwide are Finnish. Mulibrey nanism is characterized by prenatal-onset growth failure, typical facial features and pericardial constriction with hepatomegaly. The patients have an increased incidence of ovarian tumors and Wilms tumor. The intellectual capacity of Mulibrey nanism patients is normal.

Mutations in the *TRIM37* gene, encoding a peroxisomal protein, underlie Mulibrey nanism. *TRIM37* contains a RING-B-box-Coiled-coil domain (denominated TRIM for TRIPartite Motif) and an additional TRAF (Tumor necrosis factor Receptor Associated Factor) domain. Five truncating mutations with no evidence for genotype-phenotype correlation have previously been reported in Mulibrey nanism patients.

By using publicly available sequences and by sequencing the remaining gaps, we determined the genomic organization of the 24 exon *TRIM37* cDNA. Knowing the exon-intron junctions and using intronic primers, we then performed mutation screening in Mulibrey nanism patients and identified six novel disease-associated mutations. These mutations were absent in 190 control chromosomes studied. Five of the new mutations are truncating while the sixth is the first missense mutation reported so far in Mulibrey nanism patients. This missense mutation was shown to result in altered subcellular localization of the mutated *TRIM37* protein, further suggesting that it is pathogenic. The eleven Mulibrey nanism associated mutations identified to date are evenly distributed in the *TRIM37* gene. Initial analysis indicates no genotype-phenotype correlation in Mulibrey nanism.

**Point-of-care genetic testing: rapid detection of single nucleotide polymorphism (SNP) by competitive allele-specific short oligonucleotide hybridization (CASSOH) with immunochromatographic strip.** *Y. Matsubara<sup>1</sup>, S. Kure<sup>1</sup>, M. Hiratsuka<sup>2</sup>, M. Mizugaki<sup>2</sup>.* 1) Department of Medical Genetics, Tohoku University School of Medicine, Sendai, Japan; 2) Department of Clinical Pharmaceutics, Tohoku Pharmaceutical University, Sendai, Japan.

The clinical application of genetic information to individual healthcare requires simple and rapid identification of nucleotide changes in clinical settings. To date, numerous methods to detect single nucleotide substitutions (SNPs and mutations) have been reported. However, currently available SNP detection methods require either cumbersome laboratory procedures, such as electrophoresis, or expensive instrumentation which has been primarily developed for high-throughput analysis in human genome research. None of these procedures are readily performed in local clinical laboratories. Recently, we have devised a novel low-tech method for detecting SNPs using competitive allele-specific short oligonucleotide hybridization (CASSOH) with immunochromatographic strip (Matsubara & Kure, *Human Mutation*, in press). The method detects a SNP within 10 min after the completion of PCR by forming a visible gold-particle line on a chromatographic test strip. The procedure does not demand either sophisticated equipment or technical expertise. We further modified the method to allow direct analysis of whole blood without DNA extraction. The CASSOH method has been successfully applied to the detection of clinically important SNPs in drug metabolism (CYP2C19\*2, TPMT\*3C, ALDH2\*2, NAT2\*5, NAT2\*6, NAT2\*7, UGT1A1\*6 and mitochondrial 1555A>G) and Factor V Leiden. Also we were able to detect disease-causing mutations in medium-chain acyl-CoA dehydrogenase deficiency (ACADM/985A>G), non-ketotic hyperglycinemia (GLDC/1691G>T), glycogen storage disease type Ia (G6PC/727G>T), cystic fibrosis (CFTR/delF508), Tay-Sachs disease (HEXA/1277insTATC) and breast cancer (BRCA1/5382insC and BRCA2/6174delT). Our method would facilitate point-of-care genetic testing in local hospitals and out-patient clinics, promising potentially diverse clinical applications.

**Homozygosity-based screening with multiple locus-specific microsatellite markers toward the diagnosis of autosomal recessive retinitis pigmentosa.** *H. Kondo*<sup>1</sup>, *T. Tahira*<sup>2</sup>, *A. Mizota*<sup>3</sup>, *M. Kondo*<sup>4</sup>, *H. Hayashi*<sup>1</sup>, *K. Hayashi*<sup>5</sup>, *Y. Miyake*<sup>4</sup>, *K. Oshima*<sup>1</sup>, *K. Hayashi*<sup>2</sup>. 1) Ophthalmology, Fukuoka University, Fukuoka, Fukuoka, Japan; 2) Div. Genome Analysis, Res. Center for Genet. Inf., Med. Inst. of Bioregulation, Kyushu University, Fukuoka, Japan; 3) Ophthalmology, Chiba University, Chiba, Japan; 4) Ophthalmology, Nagoya University, Nagoya, Japan; 5) Hayashi Eye Hospital, Fukuoka, Japan.

Retinitis pigmentosa (RP) is a group of progressive hereditary disorders of the retina. Despite the importance of identifying the causative gene of RP, diagnosis of the recessive form (arRP) is often non-definitive due to extensive gene heterogeneity. Mutations in each of the arRP genes (16 genes identified so far) are very rare. Therefore, considerable cases of arRP may be the result of the homozygosity of single ancestral founder mutations. Compared to European countries and the United States, higher frequency of consanguineous marriage is found among the older individuals in Japanese. Thus, higher rate of mutations that are homozygous by descent is expected for the responsible genes among arRP patients of this population. Taking this into advantage, we describe a systematic method to efficiently diagnose the genetics of arRP patients. Sixty-two di-, tri-, or tetra-nucleotide repeats within or near the 16 genes known to be responsible for arRP were used as markers in multiplex amplification and genotyping. Heterozygosity of locus-specific markers excluded the majority of the examined genes as candidates, and mutation screening for the genes with homozygous alleles was considered. Thirty-eight Japanese patients with isolated RP or arRP (six with consanguineous marriage) were tested. Twenty-one patients (55%) revealed one or more heterozygous alleles of all 16 genes and were excluded in the initial screen. Of the rest of 17 patients, one, two and three genes remained as candidates in six, eight and three patients, respectively. Mutations are being searched for seven candidate genes (*CNGB1*, *NR2E3*, *PDE6A*, *PDE6B*, *RHO*, *RLBP1* and *TULP1*) in nine patients. This systematic approach should facilitate detection of homozygous alleles for molecular diagnosis of arRP.

**Frequency of Connexin 26 35delG Mutation Among Kurdish Patients With Non-syndromic Sensorineural Hearing Loss in Kermanshah, the West of Iran.** *N. Mahdieh<sup>1</sup>, K. Ali-Madadi<sup>2</sup>, H. Yazdan<sup>2</sup>, A.K. Dehghan<sup>2</sup>, S. Arzhangi<sup>1</sup>, A. Ebrahimi<sup>1</sup>, S. Kazemi<sup>2</sup>, Y. Riazalhosseini<sup>1</sup>, H. Najmabadi<sup>1</sup>.* 1) Genetics Research Center, USWR University, Tehran, Tehran, Iran; 2) Genetic Counselling Center, Welfare & Rehabilitation Organization of Kermanshah-Iran.

Hearing loss is the most prevalent form of sensory impairment in humans, with approximately 1 in 1000 live births. Mutations in connexin 26 gene are the most common cause of autosomal recessive non-syndromic hearing loss (ARNHL) throughout the world. This gene is believed relevant to half of all cases of hereditary deafness. Cx26 shows diverse mutations, that one mutation occurs very frequently in Europe- the 35delG mutation. In previous study the prevalence of GJB2 mutation has been demonstrated to be around 20%. In this study our goal is to assess the frequency of 35delG in province of Kermanshah, Kurdish population. We studied 35 patients with ARNHL by ARMS-PCR. Six of them had 35delG mutation in connexin 26 gene that 2 were homozygous, 4 were heterozygous and 27 were normal for 35delG (77%). These results suggest that frequency of 35delG is lower than around 11% compared to the other Iranian populations.

**A novel missense mutation in the Presenilin-1 (PSEN1) gene associated with sporadic Early Onset Alzheimer Disease (EOAD).** *M. Liguori<sup>1</sup>, R. Cittadella<sup>1</sup>, I. Manna<sup>1</sup>, A. La Russa<sup>1</sup>, V. La Bella<sup>2</sup>, F. Piccoli<sup>2</sup>, A. Quattrone<sup>1,3</sup>.* 1) Inst Neurological Sciences, National Research Council, Cosenza, Italy; 2) Inst Neuropsychiatry, University of Palermo, Italy; 3) Inst Neurology, University of Catanzaro, Italy.

AD is the most common cause of dementia in which genetic factors play a substantial role. The majority of EOAD has been associated to mutations in the PSEN1 gene (18 up to 50 percent), in familial and in sporadic cases. We report a novel mutation in an Italian 48-year-old woman from Sicily with a negative family history for dementia. She was a teacher but she had lost her working ability because of a progressive short-term memory impairment started 3 years before. At the time of the clinical evaluation, she had a severe cognitive decline with a temporal-spatial disorientation; she had mostly lost the ability to take care to herself. The blood laboratory parameters were normal as the analysis of her cerebrospinal fluid; MRI scan showed a diffuse cerebral atrophy. Amplifications of PSEN1 exons from 3 to 12 were performed on genomic DNA by standardised laboratory protocols. PCR products were then screened by Denaturing High-Performance Liquid Chromatography (DHPLC) technology, an automated method for detecting DNA sequence variants (Transgenomics). The eventually aberrant exons were sequenced on an ABI Prism 377 automated sequencer applying the dideoxy-dye terminator technique. Results: The DHPLC analysis showed an aberrant exon 5; the sequencing evaluation revealed a single nucleotide substitution at codon 116 (nucleotide 595: C->T, in heterozygous state) that changed the correspondent amino acid (Thr to Ile). In order to confirm this novel mutation, we analysed 50 healthy controls from the same geographic area (north-western Sicily) and 50 from another southern Italy region (Calabria) by DHPLC procedure but we did not find abnormal results in all the examined exon 5. Conclusions: These results confirm the detection of a novel T116I missense mutation in an extremely interesting region of PSEN1 gene (TM1-TM2 loop); we also confirm the feasibility of DHPLC as screening procedure for genetic point mutations.

**Notch3 mutations in families with Cadasil from southern Italy.** *R. Mazzei<sup>1</sup>, F.L. Conforti<sup>1</sup>, T. Sprovieri<sup>1</sup>, M. Zappia<sup>2</sup>, F. Bono<sup>2</sup>, A. Magariello<sup>1</sup>, A. Toscano<sup>3</sup>, A. Patitucci<sup>1</sup>, M.G. Arena<sup>3</sup>, P. Valentino<sup>2</sup>, A.L. Gabriele<sup>1</sup>, P.L. Lanza<sup>4</sup>, M. Muglia<sup>1</sup>.* 1) ISN, CNR, Mangone, Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro, Italy; 3) Department of Neuroscience, Psychiatry and Anesthesiology Science, University of Messina, Italy; 4) Neuroradiology Unit, Ospedale Annunziata, Cosenza, Italy.

CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) is an increasingly recognized adult-onset autosomal dominant vascular dementia, caused by highly stereotyped mutations in Notch3 gene on chromosome 19. Previous studies aimed at determining the distributions of mutations in the Notch3 gene found that the mutations were located in the epidermal growth factor (EGF) repeats, which are encoded for the first 23 exons but exon 1. It has been reported that 60 to 70% of mutations cluster in exons 3 and 4 and therefore a limited screen can be achieved by examining these exons alone. The aim of the present study was to screen for the exons 2-23 of Notch3 gene in 25 subjects from 7 unrelated families with CADASIL from southern Italy. All the patients were clinically examined and underwent neuroimaging studies. Molecular investigation was performed by DHPLC analysis and subsequently direct sequencing. We found 7 pathogenic mutations: two of these mutations were in exon 3, two in exon 6, three in exon 8. One mutation in exon 3 and three mutations in exon 8 are novel mutations. An optimal approach toward mutational screening in a given population is possible if the spectrum of mutations in that population is known. To date, no molecular study has been carried out for CADASIL in geographically defined populations, with the exception of Finland. Our results demonstrate that exon 4 is not the hot spot for mutations in Notch3 gene in families with CADASIL from southern Italy, and suggest that in our population can be appropriate to start molecular analysis of patients with possible CADASIL by investigating exon 8, and if this is negative to proceed to exons 3 and 6, and then to screen the remaining exons of the Notch3 gene.

**PTPN11 mutations in Noonan syndrome and hydrops fetalis.** *J.R. Jones<sup>1</sup>, T.C. Wood<sup>1</sup>, M.A. Whalen<sup>2</sup>, C. Haddock-Bolt<sup>1</sup>, L.H. Seaver<sup>1</sup>, R.J. Schroer<sup>1</sup>, R.C. Rogers<sup>1</sup>, D.E. Everman<sup>1</sup>, M.J. Friez<sup>1</sup>.* 1) Greenwood Genetic Center Greenwood, SC; 2) Boston University School of Medicine, Center for Human Genetics, Boston MA.

Noonan Syndrome (NS) is an autosomal dominant condition characterized by short stature, webbing of the neck, pectus excavatum/carinatum, cryptorchidism, characteristic facies, and cardiac defects including pulmonic stenosis and hypertrophic cardiomyopathy. NS has also been associated with hydrops fetalis. It has recently been reported that approximately 50% of NS cases may be due to mutations in the PTPN11 gene that encodes the nonreceptor protein tyrosine phosphatase SHP-2. As many as 80% of these changes have been found to cluster in specific regions of exons 3, 8, and 13. In order to identify potential PTPN11 mutations in 15 individuals with suspected NS and 12 fetuses with hydrops fetalis, we sequenced these three exons (3,8,13). Our analyses revealed mutations in 3 of the 15 patients believed to have NS. Of these three patients, two bore the diagnosis of NS based on their clinical presentation. One carried a novel A1517C transversion (Q506P) while the other possessed a previously described mutation, A1510G (M504V). The third patient presented with short stature and pulmonic stenosis; however, the case was complicated by the occurrence of congenital syphilis and hydrops. A G1507C transversion (G503R) was detected in this patient. This mutation was recently described in an individual with growth retardation, pulmonic stenosis and juvenile myelomonocytic leukemia (JMML). Our analyses also detected a G214A transition (A72T) in one female stillborn fetus described as hydropic based on the presence of subcutaneous edema and a cystic hygroma. The fetus was also noted to have a Dandy-Walker malformation, a left sided diaphragmatic hernia, an interrupted aortic arch, ventriculoseptal defect and persistent left superior vena cava. The A72T substitution has been found previously in 2 patients with JMML but without NS. Our study has identified a novel PTPN11 mutation in an individual with NS and, to our knowledge, is the first report of a PTPN11 mutation in a fetus with hydrops fetalis.

**Sodium channel -1 subunit mutations in severe myoclonic epilepsy of infancy and infantile spasms.** *J. Mulley<sup>1</sup>, R. Wallace<sup>1</sup>, B. Hodgson<sup>1</sup>, B. Grinton<sup>2</sup>, R. Gardiner<sup>3</sup>, R. Robinson<sup>3</sup>, V. Rodriguez-Casero<sup>7</sup>, L. Sadleir<sup>4</sup>, J. Morgan<sup>5</sup>, L. Harkin<sup>1</sup>, L. Dibbens<sup>1</sup>, T. Yamamoto<sup>1</sup>, E. Andermann<sup>6</sup>, S. Berkovic<sup>2</sup>, I. Scheffer<sup>2</sup>.* 1) Cytogenetics & Molec Genetics, Women's & Children's Hosp, Adelaide, Australia; 2) Medicine (Neurology), University of Melbourne, Austin & Repatriation Medical Centre, Melbourne, Australia; 3) Pediatrics & Child Health, Royal Free & University College, London, England; 4) Pediatrics, Wellington School of Medicine, University of Otago, Wellington, New Zealand; 5) Royal Children's Hospital, Llantrisant, Wales; 6) Neurogenetics, Montreal Neurological Institute and Hospital, Montreal, Canada; 7) Neurology, Royal Children's Hospital, Melbourne, Australia.

Mutations in SCN1A, the gene encoding the -1 subunit of the sodium channel, have been found in Severe Myoclonic Epilepsy of Infancy (SMEI) and Generalized Epilepsy with Febrile Seizures Plus (GEFS+). Mutations in SMEI include missense, nonsense and frameshift mutations more commonly arising *de novo* in affected patients. This finding is difficult to reconcile with the family history of GEFS+ in a significant proportion of patients with SMEI. Infantile spasms (IS) or West syndrome is a severe epileptic encephalopathy which is usually symptomatic. In some cases no etiology is found and there is a family history of epilepsy.

We screened SCN1A in 24 patients with SMEI and 23 with IS. Mutations were found in 8 of 24 (33%) SMEI patients, a frequency much lower than initial reports from Europe and Japan. One mutation near the carboxy terminus was identified in an IS patient. A family history of seizures was found in 17 of 24 patients with SMEI. The rate of SCN1A mutations in this cohort of SMEI patients suggests that other factors may be important in SMEI. Less severe mutations associated with GEFS+ could interact with other loci to cause SMEI in cases with a family history of GEFS+. This study extends the phenotypic heterogeneity of mutations in SCN1A to include infantile spasms.

**Identification of a novel WISP3 mutation in an inbred Israeli Arab kindred with progressive pseudorheumatoid dysplasia.** *Y. Hujeirat<sup>1</sup>, S. A. Shalev<sup>1</sup>, J. A. Martignetti<sup>2</sup>, R. J. Desnick<sup>2</sup>, A. Shalata<sup>2</sup>.* 1) Genetic Inst, HaEmeck Medical Ctr, Afula, Israel; 2) Human Genetic Department, Mount Sinai School of medicine, USA.

Progressive pseudorheumatoid dysplasia (PPD) (MIM# 208230) is an autosomal recessive disorder that may be initially misdiagnosed as juvenile rheumatoid arthritis. PPD is a progressive hereditary arthropathy, leading to restricted mobility of multiple peripheral joints and osseous swelling of the interphalangeal and other joints, platyspondyly and irregularities of the vertebral bodies. Symptoms appear in early childhood, and consist of limited motion, stiffness, painless swelling of several small and large joints, muscular weakness, and consequently progressive difficulty in ambulation. PPD is a rare disorder, which has been reported with increased frequency among Arab and Mediterranean populations. Mutations in the WISP3 gene, which is a member of the CCN family encoding putative growth regulators, have been found in PPD -affected individuals. We report in a newly identified large Muslim Israeli-Arab family with 8 affected individuals. Sequencing all 5 coding exons of the WISP3 gene revealed a mutation in exon 3, codon 179 which resulted in a dinucleotide deletion (del TG) leading to early protein truncation, missing the third and fourth domains, the throbospondin and c-terminal cystine knot- like domain, respectively. This homoallelic mutation was found in all affected individuals and segregated appropriately within other family members. Of the particular interest, screening of 69 unrelated healthy individuals from this village revealed an overall carrier frequency of 5.7%.

**Update on the spectrum of *TRPS1* mutations in the tricho-rhino-phalangeal syndrome types I and III. H.-J. Lüdecke, S. Gross, M. Trommler, P. Brega.** Humangenetik, Universitätsklinikum, Essen, Germany.

Mutations in the *TRPS1* gene, which codes for a zinc finger transcription factor, lead to the development of the tricho-rhino-phalangeal syndrome (TRPS) types I and III. They are characterized by craniofacial dysmorphism, brachydactyly and short stature. The latter signs are more severe in TRPS type III. Previously, we described 35 different mutations in the *TRPS1* gene (Lüdecke *et al.*, 2001, AJHG 68:81-91). Nonsense mutations were associated with TRPS I and missense mutations, exclusively found in the GATA-type zinc finger (GATA-zf, amino acids 896 to 920), caused TRPS III. Here we report the identification of 26 novel *TRPS1* mutations. Together with 7 mutations reported by others, 68 different mutations have now been found in 95 unrelated patients. Twenty of the 35 base substitutions as well as the 12 insertions and the 21 deletions lead to premature translation stop codons. Three base substitutions affect splice signals. The remaining 12 base substitutions cause missense mutations. Worth noting is that 28 of the 95 patients (29.5 %) have mutations in the 123 bp exon 6, which encodes the GATA-zf, or the adjacent intron 6 splice donor site. We found 9 novel mutations in exon 6. Two insertions, 1 deletion and 3 base substitutions cause premature stop codons. The other 3 base substitutions cause missense mutations. As expected, the W907R causes TRPS III. However, the novel A919V mutation causes TRPS I, whereas exchange of the same amino acid to threonine (A919T) resulted in a TRPS III phenotype. Interestingly, the Y923H mutation, adjacent to the GATA-zf, was found in a patient with an atypical face and normal height, but apparently small hands. Radiologic examination of the hands is pending. Surprisingly, one nonsense mutation in exon 6 (Y915X) is associated with a TRPS III phenotype. Only 4 missense mutations are not located in exon 6. Two of them (R952C and R952H) affect the nuclear localization signal and prevent nuclear entrance of TRPS1. The other two (C1217R and C1217Y) alter the first cysteine of the first IKAROS-like zinc finger. All 6 patients in whom we identified the last 4 missense mutations present with a TRPS I phenotype.

**A novel MID1 mutation in siblings with X-linked Opitz G/BBB syndrome.** *S.E. Harris<sup>1</sup>, E.V. Bawle<sup>2</sup>, E.F. Rappaport<sup>1</sup>, B.S. Emanuel<sup>1</sup>, S.C. Saitta<sup>1</sup>.* 1) Division of Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Division of Genetics and Metabolic Disorders, Children's Hospital of Michigan, Detroit, MI.

Opitz G/BBB syndrome involves midline structural defects of the craniofacies, trachea, heart and urinary tract. The phenotype is variable, but often includes hypertelorism, cleft lip and palate, tracheal and pharyngeal malformations, cardiac structural defects, and hypospadias. Both X-linked and autosomal dominant inheritance have been described. Additionally, there is significant clinical overlap with the 22q11.2 deletion syndrome. We report a family with 3 siblings (2 males and one female) with varying features of Opitz G/BBB, mildest in the affected sister. FISH analysis for a deletion of chromosome 22q11.2 was normal. Thus, despite the presence of an affected female, we examined the MID1 gene on Xp22. MID1 protein interacts with microtubules and its loss of function is associated with the X-linked form of Opitz syndrome. Using DHPLC (WAVE) analysis and confirmatory sequencing of PCR products, we detected a novel nonsense mutation in codon 575 (TGG to TGA; W575X) resulting in the loss of the last 92 amino acids of the carboxy-terminal domain. This mutation is predicted to significantly impair MID1 function, and was not found in 100 control X chromosome alleles tested. This finding underscores the importance of mutation analysis for accurate recurrence risk counseling in disorders that are genetically heterogeneous, particularly in X-linked disorders in which female heterozygotes manifest symptoms.

**A novel mutation within ECM1 gene in Korean patient with lipoproteinosis.** *J.S. Lee<sup>1, 2</sup>, C.H. Lee<sup>2</sup>, H.W. Lee<sup>2</sup>, E.S. Park<sup>2</sup>*. 1) Dept Clinical Genetics, Yonsei Univ Col Medicine, Seoul, Korea; 2) Brain Korea 21 Project for Medical Science, Yonsei University.

Lipoproteinosis(LP. OMIM 247100), also known as hyalinosis cutis et mucosae is a rare autosomal recessive disorder characterized by early hoarseness, unusual skin eruption and itchy eyes. It occurs throughout the world and more frequent in Northern Cape Province in South Africa. Genome-wide linkage analysis had recently been performed to identify a responsible gene and the gene was mapped to chromosome 1q21.2. Finally, extracellular matrix protein 1(ECM1) gene was noted as a candidate gene for the disease and different mutations were identified in patients families with different ethnic origins. ECM1 has been thought to have a role as a negative regulator of endochondral bone formation, inhibiting alkaline phosphatase activity and bone mineralization. Until today, only some mutations have been known as causes of the disease and this is the first report on a Korean patients family. We have screened exon 6 and 7 of the ECM1 gene as previously described (Hamada et al.,2002) that is known to be a hot spot for mutations in lipoproteinosis patient. Compound heterozygote mutations, ACG130ATG/892delC were identified in Korean patient. An A to T substitution changes arginine codon for methionine and 892delC mutation was previously described in a Japanese patient. Since the disease itself is very rare and only a small number of mutations were so far identified in different ethnic groups, there should be more study on the characterization of different mutations of the ECM1 gene in patients with different ethnic origins.

***OPAI* mutations in Finnish families with dominant optic atrophy (DOA).** A. Puomila<sup>1</sup>, R. Paananen<sup>1</sup>, M. Mäntyjärvi<sup>2</sup>, E.M. Sankila<sup>3</sup>, M. Somer<sup>4</sup>, M.L. Savontaus<sup>1</sup>, E. Nikoskelainen<sup>2</sup>, K. Huoponen<sup>1</sup>. 1) Department of Medical Genetics, University of Turku, Turku, Finland; 2) Department of Ophthalmology, University of Turku, Turku, Finland; 3) Helsinki University Eye Hospital, Helsinki, Finland; 4) Family Federation of Finland, Helsinki, Finland.

Several linkage studies localise dominant optic atrophy (DOA) to chromosome 3 (3q28) and mutations in the *OPAI* gene encoding a large dynamin-related GTPase protein have been detected in DOA patients. We are in the process of identifying Finnish DOA families and screening the *OPAI* gene in order to find the pathogenic mutations. At the moment, our 15 index cases include patients from large families showing linkage to the *OPAI* gene locus, as well as sporadic cases. We have used intronic primers to amplify and sequence 28 coding exons of the *OPAI* gene. Thus far, five novel pathogenic mutations have been identified in seven families. Three families share the same mutation (970delCGTTCTCCA) suggesting that they most probably have a common ancestor. The other mutations are restricted to one family only, as seen in other populations. Different types of mutations were found (703CT ArgSTOP, 1065+2tc, 1212delTgtaa, 1768CT ArgTrp). Mutation screening of the other cases is still going on. As there seems to be no clear mutational hot-spot in the *OPAI* gene, simple rapid DNA testing can be offered only to those families where the mutation is known.

**The recurrent Basque mutation in Brazilian calpainopathy patients.** *F. Paula<sup>1</sup>, A. Sáenz<sup>2</sup>, A.M. Cobo<sup>2</sup>, A. Lopez de Munain<sup>2</sup>, A. Urtizberea<sup>3</sup>, M. Zatz<sup>1</sup>.* 1) Human Genome Research Center, Sao Paulo University, Sao Paulo, Brazil; 2) Hospital Donostia, San Sebastián, Basque Country, Spain; 3) Service de Medecine Physique et Readaptation de l'Enfant, Hospital Raymond Poincare, Garches, France.

Limb-girdle muscular dystrophy (LGMD) is a heterogeneous group of genetic disorders characterized by progressive weakness predominantly in the pelvic and shoulder-girdle musculature. LGMD2A, one of the most prevalent forms of LGMD in many populations, is caused by mutations in the calpain-3 gene (CAPN3). More than 120 CAPN3 mutations have been identified. Most represent private changes found in specific families but, particular pathogenic changes have been reported more frequently in some genetic isolates. One of this, the recurrent 2362AG>TCATCT mutation (exon 22), was found in more than 90% of the calpainopathy patients from Basque country, most of them associated with a founder haplotype. We have recently identified pathogenic mutations in the CAPN3 gene in patients from 44 unrelated Brazilian genealogies. Eleven families (25%) were found to carry the 2362AG>TCATCT change: 6 are homozygotes and 5 are compound heterozygotes. In order to verify the origin of this mutation in Brazilian patients we are studying the haplotype flanking the CAPN3 gene as compared to one Basque patient using 4 microsatellites with 1cM interval: cen-D15S779-D15S782-D15S778-D15S783-ter. All 2362AG>TCATCT Brazilian chromosomes have the same haplotype (1, 1, 4, 3) suggesting a single origin for this mutation. We are currently comparing these results with Basque patients carrying the same mutation. The preliminary results showed in one Basque patient the same haplotype suggesting a common origin for the 2362AG>TCATCT mutation in Brazilian and Basque patients. Since immigrants from several European countries, including Portugal and Spain, colonized Brazil it is likely that the origin of this mutation is Basque. Supported by FAPESP-CEPID, PRONEX, CNPq.

**Mutation analysis of the L1CAM gene in 200 patients.** *Y.J. Vos<sup>1</sup>, E. Verlind<sup>1</sup>, A. Kooistra<sup>1</sup>, I. Stolte-Dijkstra<sup>1</sup>, W.S. Kerstjens-Frederikse<sup>1</sup>, C.H.C.M. Buys<sup>1</sup>, R.M.W. Hofstra<sup>2</sup>.* 1) Clinical Genetics, University Hospital, Groningen, The Netherlands; 2) Medical Genetics, University of Groningen, The Netherlands.

Mutations in the L1CAM gene, which encodes an immunoglobulin-like neural cell adhesion molecule, cause several neurological syndromes: HSAS (Hydrocephalus due to Stenosis of the Aqueduct of Sylvius); MASA syndrome (Mental Retardation, Aphasia, Shuffling gait, adducted thumbs); SP1 (Spastic Paraparesis type 1); ACC (Agenesis of Corpus Callosum). The L1 gene is located at Xq28, consists of 28 exons and encodes a protein of 1256 amino acids. The L1-protein is a transmembrane glycoprotein. It includes an extracellular part with six immunoglobulin like domains and five fibronectin type III like domains, a transmembrane domain and a short cytoplasmic C-terminal tail. Up to now 142 different pathogenic mutations, in 153 families, spread over all regions of the gene, have been identified and published (<http://dnalab-www.uia.ac.be/dnalab/11/>). The clinical diagnosis of a male patient is based on the finding of two or more characteristics such as hydrocephalus and adducted thumbs, or spasticity and mental retardation. In the majority of cases there is a positive family history. Confirmation of the clinical diagnosis by molecular analysis is essential to identify carriers in the families and to make it possible to offer reliable prenatal diagnosis. Since the molecular analysis of the L1CAM gene is very laborious, because of the rather large size of the coding region, we developed an efficient pre-screening method, based on denaturing gradient gel electrophoresis (DGGE), followed by direct sequencing of the amplicon with an aberrant banding pattern. To date DNA of 200 patients has been analysed, patients with a positive family history as well as sporadic cases. In 39 patients, 36 different mutations have been found: 13 missense mutations, 7 nonsense mutations, 6 deletions/insertions, 9 splice site mutations and in one case the deletion of the whole gene. Of those mutations 24 had not been published before. Most missense mutations are considered pathogenic as they affect the structural integrity of one of the above mentioned domains.

**Mutations in the *SIMPLE* gene are a relative common cause of dominant Charcot-Marie-Tooth disease (CMT1C) and related peripheral neuropathies.** *G.M. Saifi*<sup>1</sup>, *K. Szigeti*<sup>1</sup>, *W. Wiszniewski*<sup>1</sup>, *J.R. Lupski*<sup>1,2</sup>. 1) Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Room 604B, Houston, TX 77030, USA; 2) Department of Pediatrics, Baylor College of Medicine, One Baylor Plaza, Room 604B, Houston, TX 77030, USA.

Charcot-Marie-Tooth disease is a clinically and genetically heterogeneous group of inherited peripheral neuropathies characterized by progressive weakness and atrophy of distal limb muscles. It has an estimated prevalence of 1 in 2500. Mutations have been reported in twenty genes and an additional ten loci are associated with CMT. We report here the results of our studies on one of the recently identified CMT genes, *SIMPLE*. We screened this gene for mutations in 192 CMT cases. In these 192 cases, a molecular etiology has not been established despite examining for many potential causes in several CMT genes including the CMT1A duplication, *PMP22*, *Cx32*, *MPZ*, *EGR2*, *PRX*, *NEFL*, *MTMR2*, *GDAP1*, *TDPI*, *SOX10* and *LMNA*. We have so far identified four different missense mutations in *SIMPLE* in nine families. In addition, we identified two silent changes in another nine CMT families. The missense mutations and the silent changes were not found in 188 control chromosomes. It thus appears that mutations in *SIMPLE* could be the most frequent cause of CMT after the CMT1A duplication and *Cx32* mutations. Segregation analysis has confirmed that one of the mutations is associated with the disease phenotype. Out of these 18 families, there are 3 families in whom we had previously identified mutations in other CMT genes and the mutation had not segregated with the disease phenotype. We are currently screening these families for simultaneous segregation of one of the silent changes in *SIMPLE* and the mutation identified in one of the other CMT genes. Further, preliminary studies indicate that the phenotypes of patients fall in a wide spectrum, similar to what has been observed in *MPZ* mutations. This indicates that mutations in *SIMPLE* in conjugation with a mutation in another CMT gene may be central to the pathogenesis of the disease and modulate the variability of the phenotype.

**Sequence alterations in the RP1 and RHO genes in Chinese patients with retinitis pigmentosa.** *C.P. Pang, D.Y. Wang, P.O.S. Tam, L. Baum, K.W. Choy, D.S.C. Lam, W.M. Chan.* Ophthalmology & Visual Sci, Chinese Univ Hong Kong, Hong Kong, China.

Retinitis pigmentosa (RP) is among the most common inherited forms of blindness, affecting about 1 in 4,000 people in most ethnic groups. Mutations in many genes cause RP. We screened for sequence alterations in the coding exons and splice regions of the RHO and RP1 genes in 173 unrelated Chinese patients, some of their family members, and 190 control subjects. Among 10 sequence changes found in RHO, M309I, P347L and 5211delC caused RP. 5211delC leads to the negatively-charged final 22 amino acids containing 6 phosphorylation sites being replaced by a 32 amino acid positively-charged nonsense sequence. In RP1 we identified 2 nonsense and 11 missense sequence alterations: R677X and D984G caused RP and R1933X did not. In this Chinese RP population 6 RHO and 10 RP1 novel sequence variations were found. In contrast to the other C-terminal truncation, Y1053, R1933X of RP1 appears benign. Therefore the RP1 polypeptide sequence between amino acids 1052 and 1933, which contains a transmembrane domain (1120-1142) and leucine-zipper motif (1396-1417), must be present at undiminished levels to maintain photoreceptor function. In RHO, the C-terminal nonsense mutation 5211delC may lead to mis-sorting of rhodopsin protein. The expected frequency of RP1 and RHO mutations combined among this Chinese RP population is low, less than 10% ( $5/173=3.0\%$ , 95% confidence interval: 0.4%-10.0%).

**Development of a molecular diagnostic screening for the autosomal recessive polycystic kidney disease gene,**

***PKHD1***. S. Rossetti<sup>1</sup>, R. Torra<sup>2</sup>, E. Coto<sup>3</sup>, M. Consugar<sup>1</sup>, V. Kubly<sup>1</sup>, M. El-Youssef<sup>1</sup>, V.E. Torres<sup>1</sup>, P.C. Harris<sup>1</sup>. 1) Div Nephrology and Gastroenterology, Mayo Clinic & Foundation, Rochester, MN; 2) Fundacio' Puigvert, Barcelona, Spain; 3) Hospital Central de Asturias, Oviedo, Spain.

Autosomal Recessive Polycystic Kidney Disease (ARPKD) is a severe form of childhood nephropathy affecting about 1:20,000 live births. The disease is characterized by fusiform dilatation of the collecting ducts in the kidney and congenital hepatic fibrosis (CHF) and Caroli's disease, (CD) in the liver. The ARPKD gene, *PKHD1*, has been recently cloned and contains 66 coding exons that encode a ~450 Kda protein, fibrocystin. We have used denaturing high-performance liquid chromatography for mutation analysis of this large multi-exon gene in a large cohort of 89 pedigrees, 64 with a primary diagnosis of ARPKD and 25 with CHF and/or CD. This phenotypically heterogeneous cohort has allowed us to better define the phenotypic range associated with *PKHD1* mutations. A total of 47 different mutations were found on 78 disease alleles. The detection rate was highest in severely affected ARPKD patients (perinatal death)(85%), 42% in other ARPKD cases and lowest (32%) but still important in CHF/CD. These mutations were truncating, missense and potential splicing changes, and both mutations were identified in 27 pedigrees, plus one mutation in additional 24 families. Some frequent mutations (9689delA, 5895InsA, T2869K, T36M and I222V) were proven to be ancestral changes by haplotype analysis with existing and newly developed intragenic and closely flanking microsatellite markers. Preliminary genotype-phenotype correlations show that probands with a double-truncating genotype have the severe form of ARPKD. The modest detection rate in ARPKD cases without the most severe disease indicates that an accurate description of the phenotype may be important. Genotyping analysis using a haplotype generated with the new highly informative and closely linked markers could complement the mutation screening in a diagnostic setting, in particular in families in which one diagnostic *PKHD1* mutation has been identified. Overall, the prospect for molecular genetic testing in this devastating disorder looks good.

**L1 retrotransposition during embryogenesis in the mother of a patient with choroideremia.** *J.A.J.M. van den Hurk<sup>1</sup>, I.C. Meij<sup>1</sup>, L.H. Hoefsloot<sup>1</sup>, I.J. de Wijs<sup>1</sup>, T.J.R. van de Pol<sup>1</sup>, A.S. Plomp<sup>2</sup>, H.G. Brunner<sup>1</sup>, F.P.M. Cremers<sup>1</sup>.* 1) Department of Human Genetics, University Medical Center Nijmegen, Nijmegen, The Netherlands; 2) The Netherlands Ophthalmic Research Institute, Amsterdam, The Netherlands.

Choroideremia (CHM) is a progressive chorioretinal degeneration caused by mutations in the widely expressed *CHM* gene on chromosome Xq21. The product of this gene, Rab escort protein (REP)-1, is involved in the posttranslational lipid modification and subsequent membrane targeting of Rab proteins, small GTPases that play a key role in intracellular trafficking.

In the course of a comprehensive mutation screen of the *CHM* gene, an insertion of a full-length L1 retrotransposon into *CHM* exon 6 was identified in a patient with choroideremia. The L1 insertion causes the deletion of exon 6 from the *CHM* mRNA. As the reading frame is maintained, this results in a protein product lacking amino acids 235 through 273. Interestingly, analyses of the patients family indicated germline and somatic mosaicism for the L1 element in the *CHM* gene in the mother. These findings provide the first evidence consistent with an L1 retrotransposition event during embryogenesis.

**Mutations of the PKD1 gene among Japanese patients with autosomal dominant polycystic kidney disease. T.**

*Tamura*<sup>1</sup>, *A. Yamaki*<sup>1</sup>, *S. Hashimoto*<sup>1</sup>, *E. Higashihara*<sup>2</sup>, *Y. Shimizu*<sup>1</sup>. 1) Dept Medical Genetics, Kyorin Univ Sch Health Sci, Hachioji, Tokyo, Japan; 2) Dept Urology, Kyorin Univ Sch Medicine, Mitaka, Tokyo.

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common hereditary disorders. The prevalence of ADPKD in the Japanese population has been estimated as approximately 1 in 4000. We performed the linkage analysis using six polymorphic microsatellite markers flanking to PKD1 or PKD2. Among 21 Japanese ADPKD families, 16 families linked to PKD1 and 2 families linked to PKD2 whereas 3 families (K68, K71, K123) linked to neither PKD1 nor PKD2. However, two different nonsense mutations were detected in two of three affected members of families K68 and K123. Mutation detection of PKD1 has been complicated because several PKD1-like genes locate on chromosome 16p13.1 and 2.5 kb polypyrimidine tract is present in intron 21 of PKD1 gene. Although more than 100 mutations such as various amino acids alterations and abnormal splicing have been reported for entire PKD1 gene, hot spots were not yet found. We performed simple PCR for the unique region (exon 34 ~ 46) and long range PCR followed by short range PCR for the homologous region (exon 22 ~ 33). We analyzed PCR products by WAVE (DHPLC method). We found eight mutations in the unique region: one splicing mutation caused by 20 bp deletion in intron 43, C>A at 48566 (Cys3693Ter) in exon 38, C>T at 51237 (Gln4124Ter) and C>T at 51042 (Glu4059Ter) in exon 45, G deletion at 48012 in exon 37, GGTC insertion at 50868 in exon 44, CC deletion at 51114 and GCACCTG insertion at 51110 in exon 45. In the homologous region seven missense mutations were detected and these mutations were not found in 50 unrelated normal individuals. 26 SNPs including 3 cSNPs. were found during the course of PKD1 mutant detection.

**Identification of a novel SCA14 mutation in Dutch autosomal dominant cerebellar ataxia families.** *R.J. Sinke<sup>1</sup>, D.S. Verbeek<sup>1</sup>, B.P.C. van de Warrenburg<sup>2</sup>, S.J. Piersma<sup>1</sup>, F.A.M. Hennekam<sup>1</sup>, P.F. Ippel<sup>1</sup>, P.L. Pearson<sup>1</sup>, N.V.A.M. Knoers<sup>3</sup>, H.P.H. Kremer<sup>2</sup>.* 1) Dept. of Medical Genetics, University Medical Center Utrecht, Utrecht, Netherlands; 2) Dept. of Neurology, University Medical Center Nijmegen, Nijmegen, The Netherlands; 3) Dept. of Human Genetics, University Medical Center Nijmegen, Nijmegen, The Netherlands.

Autosomal dominant cerebellar ataxias (ADCAs) comprise a group of neurodegenerative disorders, characterized by an adult age of onset, gait and limb ataxia, disturbances of speech and oculomotor control, and additional variable features. To date, 22 different SCA (spinocerebellar ataxia) loci have been identified in ADCA. The corresponding gene has been cloned for ten of these loci. Screening of the SCA1,2,3,6 and 7 genes has yielded a diagnosis for about 70% of the Dutch ADCA families. Mutations in the SCA10,12 and 15 genes have not been detected in the remaining families. Interestingly, the CAG repeat expansions, especially in the SCA3 and SCA6 genes, display strong founder effects in the Dutch ADCA population. Because this might also be the case for other, unknown SCA genes, we searched for shared haplotypes in 28 small Dutch ADCA families to try to identify novel SCA genes. We initially set out to analyze all reported SCA-loci for which the corresponding genes had not yet been identified. In addition, we performed genealogical investigations to link any distantly related families. By doing so, we identified 3 small families that appeared to be related and that could be linked to the SCA14 locus on chromosome 19 (max. lod score 4,62). At this stage, the PRKCG gene was identified as the SCA14 disease gene. Accordingly, we sequenced this gene in the affected family members and identified a novel missense mutation, Gly118Asp, in exon 4. Currently, we are screening the entire Dutch SCA negative panel for additional SCA14 mutations. While the mutation mechanism in most SCA genes is identified as repeat expansions, in the two most recent SCA genes (SCA14 and FGF14) missense mutations appear to be pathogenic. This opens up a new perspective in the further unraveling of the intriguing disease mechanism of these neurodegenerative disorders.

**Two novel mutations in the PTPN11 gene associated with Noonan and LEOPARD syndromes.** *S. Ylonen<sup>1</sup>, J. Körkkö<sup>1</sup>, M. Somer<sup>2</sup>, J. Ignatius<sup>1</sup>.* 1) Dept of Clinical Genetics, University Hospital of Oulu, OULU, FINLAND; 2) Dept of Clinical Genetics, Väestöliitto, The Finnish Family Federation, HELSINKI, FINLAND.

Recently, missense mutations in PTPN11, the gene encoding protein-tyrosine phosphatase, nonreceptor type,11, has been described in several patients with Noonan syndrome (NS) or Multiple Lentigines /LEOPARD syndrome (LS). Some of these mutations have been described in more than one family. Codon 308 in exon 8 appears to be a significant mutation hotspot for NS, Asn308Asp being the most common mutation found in several studies. That codon 308 is a hotspot for NS has been further indicated by the finding of an Asn308Ser missense mutation in some additional families. In LS, hotspots have been found in exons 7 and 12. We have investigated PTPN11 gene mutations with PCR-CSGE-sequencing and gene sequencing in 4 Finnish NS patients and in one two generation family with prominent lentigines and the diagnosis consistent with LS. In one patient with typical NS we found an Asn308Thr mutation which has not been described before. This finding further indicates that codon 308 in exon 8 is a significant mutation hotspot in NS. In the LS family we found a novel mutation Gln510Glu in exon 13. This finding indicates that mutations causing Multiple Lentigines/LEOPARD syndrome phenotype are not restricted to exons 7 and 12.

**The C1490Y mutation in *ABCA4* ; a possible founder mutation in the South African Stargardt population.** A.V. September, R. Ramesar, L.J. Greenberg. Human Genetics, University of Cape Town, Cape Town, South Africa.

Mutations in *ABCA4* are associated with autosomal recessive Stargardt disease (STGD). This study aimed to assess the mutation spectrum of *ABCA4* underlying STGD in South Africa (SA).

Sixty one affected, unrelated STGD individuals and 120 unrelated, unaffected, ethnically matched control individuals were selected. Single strand conformation polymorphism analysis (SSCP) and Heteroduplex (HD) analysis in conjunction with polymerase chain reaction amplification was used as the mutation detection technique to screen the entire coding region of the gene, followed by direct sequencing. Haplotypes were constructed for 11 of the 61 families using the mutation and the markers D1S188, D1S406 and D1S236.

A total of 53 sequence variants were identified that were potential disease-causing mutations in 37 of the 61 STGD families. The majority of these individuals were heterozygous for single mutations with 16 of the 61 individuals found to be compound heterozygotes for 2 different mutations. These variations included single base substitutions resulting in 10 missense, 2 truncation and 2 splice site mutations. In addition, 1 deletion resulting in a frameshift mutation was also identified. Two of the 15 different sequence variants identified were novel. The C1490Y variant was the most common mutation identified (19/61) and was absent in the 120 controls. This variant has only ever been identified in 3/150 patients from North and Central Europe. A single haplotype was identified for the 4 most common sequence variants and 2 haplotypes were identified for R602W.

In summary, at least one mutation has been identified in 61% of the STGD individuals investigated of whom 26% are compound heterozygotes. The mutation analysis together with the haplotype analysis, have demonstrated that there are several origins of mutations of the *ABCA4* gene, within the SA STGD population. The observed high prevalence of the C1490Y variant together with the haplotype analysis suggests a possible founder effect in SA.

**Mutation analysis of the *NSDI* gene - genetic testing for Sotos syndrome.,** *G. Raca*<sup>1</sup>, *D.J. Waggoner*<sup>1</sup>, *J. Kamimura*<sup>2</sup>, *N. Matsumoto*<sup>2</sup>, *G.B. Schaefer*<sup>3</sup>, *K.O. Welch*<sup>1</sup>, *C.L. Martin*<sup>1</sup>, *S. Das*<sup>1</sup>. 1) Human Genetics, University of Chicago, Chicago, IL; 2) Human Genetics, Nagasaki University School of Medicine, Sakamoto, Japan; 3) Nebraska Medical Center, Omaha, NEX.

Sotos syndrome is characterized by pre and postnatal accelerated somatic growth, characteristic facies, advanced bone age and developmental delay. Deletions and mutations of the *NSDI* gene have recently been demonstrated in patients with Sotos syndrome and account for approximately 77% of patients. We have developed mutation analysis of the *NSDI* gene using a denaturing high performance liquid chromatography (DHPLC) and sequencing-based assay for clinical testing purposes. The *NSDI* gene has 23 exons that are amplified with flanking intron sequence in 47 PCRs. Amplified products are subject to DHPLC analysis using optimized temperature conditions predicted using the Navigator software program (Transgenomic Inc.). We have validated our DHPLC/sequencing-based mutation assay in the *NSDI* gene and have accurately identified all 29 sequence changes tested, including a number performed in a blinded fashion. To date we have performed deletion analysis of the *NSDI* gene by FISH testing in 118 patients referred to our laboratory for Sotos syndrome and found deletions in 7, i.e. 6%. Patients with *NSDI* deletions tended to have the typical features of Sotos syndrome. Mutations have been identified in 7 of 13 (54%) non-deleted Sotos syndrome patients as well as seven polymorphisms were identified. Two patients with Weaver syndrome were analyzed and no mutations were found. While the majority of patients with mutations had typical features of Sotos syndrome, there was clinical variability within the group. Two patients did not have advanced growth while two patients did not have dysmorphic features. We are currently in the process of analyzing further patients for mutations in the *NSDI* gene and genotype-phenotype results of these patients will be presented.

**Mutations of UMOD in the tubulo-interstitial diseases MCKD and FJHN associate with intracellular aggregates and excretion reduction of uromodulin.** *L. Rampoldi<sup>1</sup>, G. Caridi<sup>2</sup>, F. Boaretto<sup>3</sup>, I. Bernascone<sup>1</sup>, D. Santon<sup>3</sup>, G. Lamorte<sup>1</sup>, R. Tardanico<sup>4</sup>, G. Colussi<sup>5</sup>, F. Scolari<sup>4</sup>, A. Amoroso<sup>3</sup>, G. Ghiggeri<sup>2</sup>, G. Casari<sup>1</sup>.* 1) DIBIT, S.Raffaele Scientific Institute, Milan, MI, Italy; 2) Laboratory of Nephrology, G. Gaslini Institute, Genoa, Italy; 3) Genetics Service, Istituto per l'Infanzia Burlo Garofolo, Trieste, Italy; 4) Spedali Civili and University of Brescia, Brescia, Italy; 5) Ospedale di Circolo, Varese, Italy.

Medullary cystic kidney disease is a typical tubulo-interstitial disorder characterized by alteration of urinary concentration, hyperuricemia, tubulo-interstitial fibrosis, frequent cysts at the cortico-medullary junction and renal failure. It is a genetic and clinical heterogeneous disorder with two known loci, i.e. MCKD1 (OMIM 174000) on chromosome 1q21 and MCKD2 (OMIM 603860) on chromosome 16p12. Familial juvenile hyperuricemic nephropathy (OMIM 162000) features are very similar to those seen in MCKD, i.e. hyperuricemia, gout, progressive renal failure and autosomal-dominant inheritance. A locus for FJHN was mapped to a chromosome 16p region that overlapped with the MCKD2 locus in two Czech and one Japanese families. Very recently, mutations in the gene encoding uromodulin have been reported in MCKD and FJHN families, demonstrating that FJHN and MCKD are allelic disorders. In two unrelated MCKD and one FJHN Italian families linked or compatible with linkage to the chromosome 16 MCKD2/FJHN locus we performed mutational analysis of UMOD gene and identified three novel uromodulin gene mutations that segregated with the disease phenotype. These results provide the first independent confirmation that UMOD mutations are responsible for MCKD2. Immunohistochemistry and electron microscopy on MCKD/FJHN kidney biopsies reveal pathologic evidence of dense intracellular accumulation of uromodulin in tubular epithelia of the thick ascending limb of Henle's loop. Western blot analysis of patient urine shows a severe reduction of excreted uromodulin. Preliminary transfection experiments with the mutant isoforms in HEK293 and HeLa cell lines that do not express uromodulin reveal a maturation impairment of mutant protein that is consistent with clinical findings.

Program Nr: 2429 from 2003 ASHG Annual Meeting

**Characterization of novel mutations in the Tudor domain and in the promoter region of the SMN1 gene in Spanish SMA patients.** *E. Tizzano, I. Cusco, M.J. Barcelo, E. del Rio, M. Baiget.* Dept Genetics, Hosp Sant Pau, Barcelona, Spain.

Spinal muscular atrophy( is an autosomal recessive neuromuscular disorder characterized by the degeneration and loss of motor neurones of the anterior horn of the spinal cord. The absence or mutations in the survival motor neurone (SMN1) gene causes SMA resulting in a reduced production of functional SMN protein. Approximately 90% of the SMA patients present homozygous deletions of the SMN1 gene and in the remaining patients, subtle mutations have been described. We present a thorough molecular analysis of four type I SMA patients who retained one copy of the SMN1 gene. Long-range genomic PCR analysis and RNA studies led to the identification of four previously unreported changes. Two of them were intragenic mutations (I116F and Q136E)affecting a very conserved region containing the Tudor domain of the exon 3. Alteration of this highly conserved domain should be critical for SMN function in spinal cord given that both patients suffered from type I SMA and died before the first year of life. In the remaining two patients no changes were found in the coding region or exon-intron boundaries but in one a promoter point mutation was identified that led to the absence of SMN1 transcript. The last patient showed a partial deletion of the 5' end of the gene that includes the C212 and C272 markers and ends before exon 2a. Our results highlight the importance of the Tudor domain region for SMN function and indicate that SMA patients without homozygous deletion of exon 7-8 or subtle mutations in the coding sequences may have transcription defects as a result of point mutation or deletions in the promoter region. Supported by FIS 02-1275.

**Novel disease-causing mutations in DNAI1 gene in Primary Ciliary Dyskinesia (PCD).** M. Zariwala<sup>1</sup>, P.G. Noone<sup>1</sup>, M.W. Leigh<sup>1</sup>, R.U. De Iongh<sup>2</sup>, L.M. Morgan<sup>2</sup>, J. Horvath<sup>3</sup>, H. Omran<sup>3</sup>, H. Mitchison<sup>4</sup>, M.R. Knowles<sup>1</sup>. 1) UNC-Chapel Hill, NC; 2) Concord Hospital, Sydney; 3) the Universitats-Kinderklinik, Freiburg; 4) Royal Free and University College Medical School, London.

Primary ciliary dyskinesia (PCD), usually inherited as an autosomal recessive trait, is characterized by sino-pulmonary disease and half the patients have situs inversus. The PCD phenotype results from axonemal abnormalities; 80% have dynein arm defects in cilia. It is a heterogeneous disorder and disease causing mutations have been reported in *DNAH5* (n=6 families) [Olbrich et al., Nat. Genet. 30: 143, 2002] and *DNAI1* (n=6 families) [Pennarun et al., AJHG 65: 1508, 1999; Guichard et al., AJHG 68: 1030, 2001; Zariwala et al., AJRCMB 25: 577, 2001]. The 219+3insT mutation in *DNAI1* represents 6 of the 12 reported mutant alleles and 3 other mutant alleles are in exon 17 of *DNAI1*. We studied 81 unrelated PCD patients to test for 219+3insT and exon 17 mutations in *DNAI1* (41 with situs inversus). Of the 76 patients with ultrastructural analysis of cilia, 70% had defective outer dynein arm. The 219+3insT mutation was screened by mutation-induced *HpaI* restriction site; 3 of 81 patients harbored 219+3insT mutation (2 were homozygous and 1 was heterozygous). Due to allelic heterogeneity, we used sequence analyses to screen exon 17. No previously reported mutations in exon 17 were detected, but 2 patients had novel mutations at codons 538 and 548. One mutation (G1783A; A538T) was found on one allele of a patient, who had 219+3insT on the other allele. Another patient had the A538T mutation on one allele (inherited from the mother) and a nonsense mutation (G1815; W548X) on the second allele (inherited from the father). Each of these mutations resides in a conserved WD-repeat region and would be predicted to lead to abnormal folding of the protein. None of the 160 alleles analysed from non-PCD controls had the 2 novel mutations. In conclusion, we detected mutations in *DNAI1* in 4 unrelated PCD patients. This abstract is funded by GCRC #00046, M01 RR00046-42 and NIH HL04225.

**Spontaneous multiple mutations in mouse show proximal spacing consistent with temporally coordinate events and alterations with p53-deficiency.** *J. Wang, K. Hill, K. Farwell, W. Scaringe, S. Sommer.* Dept Molecular Genetics, City of Hope/Beckman Res Inst, Duarte, CA.

Analysis of spontaneous multiple mutations in normal and tumor cells may constrain hypotheses about the mechanisms responsible for multiple mutations and provide insight into the mutator phenotype. Previously, it was estimated that spontaneous multiple mutations in Big Blue mice are about 660-fold more frequent than expected by chance in both normal and tumor tissues [Buettner et al., 2000 *Mutat Res*, 252:219]. The spacing between multiple mutations was generally close; the distribution of mutation spacing fit an exponential distribution, albeit with substantial scatter. Herein, the frequency, pattern and spectrum of spontaneous multiple mutations are examined as a function of age, tissue type, p53 deficiency and neoplasia in a sample augmented by 2,658 additional, sequenced mutants. Previous findings are confirmed and several additional observations are made: i) spacing between mutations in doublets in this larger sample now fit an exponential distribution more precisely ( $R^2=0.98$ ), consistent with temporally coordinate events. The half life of mutation spacing is 120 nucleotides; ii) doublet frequencies are similar among somatic tissues and reduced in the germline; iii) singlets and doublets show similar overall increases in frequency with age; iv) doublet frequencies are elevated in somatic tissues of p53-deficient mice (Li Fraumini cancer syndrome model;  $p=0.005$ ) and v) doublets and singlets in tumors have a significantly different mutation pattern ( $p=0.007$ ). The observations are consistent with the temporally coordinated occurrence of spontaneous multiple mutations by a transient error-prone condition and do not suggest a major role for the recently discovered Y family of error-prone polymerases.

**Comprehensive Sequence Analysis Ruled out Disease Association with the CFTR Gene in a Family with Atypical CF Presentations.** *W. Sun<sup>1</sup>, J. Redman<sup>1</sup>, R. Wallerstein<sup>2</sup>, J. McCarrier<sup>2</sup>, D. Lee<sup>2</sup>, A. Buller<sup>1</sup>, M. McGinniss<sup>1</sup>, F. Quan<sup>1</sup>, C. Strom<sup>1</sup>.* 1) Molec Gen Dept, Nichols Inst, Quest Diagnostics Inc, San Juan Capistrano, CA; 2) Department of Pediatrics, Hackensack University Medical Center, Hackensack, NJ 07601.

Cystic fibrosis (CF) is the most common life-limiting recessive genetic disease in Caucasians. Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene are responsible for the disease in almost all patients. In addition to causing the classical CF phenotype, CFTR mutations have been associated with congenital bilateral absence of the vas deferens (CBAVD), chronic pancreatitis, allergic bronchopulmonary aspergillosis and chronic rhinosinusitis. Nevertheless, factors other than mutations in the CFTR gene can produce phenotypes clinically indistinguishable from nonclassic cystic fibrosis caused by CFTR dysfunction (N Engl J Med. 347:401-7). We report that comprehensive sequence analyses of the entire coding region as well as the splice junction sites of the CFTR gene have been performed on a family with atypical CF presentations, including both parents and four symptomatic children with chronic sinusitis and chronic cough. One sibling, the most seriously affected, had pan-sinusitis, bronchiectasis, and recurrent otitis. Her genotype showed homozygosity for the M470V polymorphism only. We detected two previously reported rare gene variants, G576A and R668C, in two of the affected children. However, our study indicated that these variants are in cis in this family. In addition, other polymorphic markers in the CFTR gene allowed us to track the segregation of parental CFTR alleles to each of the children. Since the affected children have inherited different CFTR alleles, our data rules out CFTR as the sole causative agent. G576A and R668C, did not cosegregate with disease phenotype and thus are not pathogenic in this family. Our study also showed that comprehensive sequence analysis of the CFTR gene not only detected rare disease-causing mutations and variants, it also served as a powerful tool for linkage study to establish/rule out disease association with the CFTR gene locus in affected families.

**Identification and characterization of novel mutations in patients with Enhanced S Cone syndrome.** *N. Udar<sup>1</sup>, M. Marmor<sup>2</sup>, M. Chalukya<sup>1</sup>, S. Yelchits<sup>1</sup>, R. Silva-Garcia<sup>1</sup>, K. Small<sup>1</sup>.* 1) Jules Stein Eye Institute, The David Geffen School of Medicine at UCLA, Los Angeles, CA; 2) Department of Ophthalmology, Stanford University Medical Center, Stanford, California.

Enhanced S Cone syndrome (ESCS) is a rare autosomal recessive retinal disorder characterized by absence of rod function and by large amplitude S cone mediated signal to short wavelength stimuli with an increased sensitivity to blue light. Unlike other inherited retinal degenerations where photoreceptors are lost due to apoptosis, ESCS manifests a gain in function of photoreceptors. The causative gene NR2E3, is a nuclear receptor located on chromosome 15q23. NR2E3 is a retinal expressed ligand-dependent transcription factor with a 2.2 kb transcript. Although there are very few reports of mutation screening for this disorder, the majority of reported mutations involve R311Q at the 3 end of the gene. In contrast, our mutations were at the 5 end of the gene. Using a direct sequencing approach, we screened the entire gene in 2 patients (1 from Brazil) belonging to independent families and identified the mutations. The first patients is a compound heterozygote for IVS1-2AC and del 190-198. The other patient was homozygous for del 196-201. Only the intronic mutation has previously been reported, the other two are novel. Interestingly the only other 9 base deletion reported previously overlaps with the mutations we have identified and is likely to occur due to a simple repeat within the exon. The identification of these mutations implies that the amino acid 64-69 are critically necessary for the proper functioning of the protein. Our results identify the second hot-spot for mutation within this gene. This is also the first report of the mutation in a Brazilian family with ESCS.

**Myogenesis in Facioscapulohumeral Dystrophy (FSHD) and Control Myoblasts.** *D.A. Figlewicz<sup>1,2</sup>, I. Coltas<sup>1</sup>, Y. Hong<sup>1</sup>, J.E. Sowden<sup>2</sup>, R. Tawil<sup>2</sup>.* 1) Neurology, University of Michigan, Ann Arbor, MI; 2) Neurology, University of Rochester, Rochester, NY.

Facioscapulohumeral dystrophy (FSHD) is an autosomal dominant disorder resulting from deletion of 3.3kb repeats on chromosome 4q35. We have observed phenotypic differences in myoblasts cultured from FSHD skeletal muscle biopsies, including morphologic changes and loss of replicative capacity. Our data to date support the hypothesis that FSHD myoblasts leave the cell cycle earlier, fuse more readily, and upregulate some genes associated with myogenesis. In order to further investigate this phenomenon we are studying the relationship between several cell cycle and transcription regulators in myoblasts (including p53, p21, MyoD, and myostatin) using real-time PCR, immunocytochemistry and Western blotting. Myostatin is a member of the TGF-beta family of growth factors and a negative regulator of myogenesis; inhibition of myostatin or myostatin signalling pathways is currently of great interest as a therapeutic treatment for muscular dystrophies. It has been proposed by others that the myostatin gene is a downstream target gene of MyoD and may be involved in myoblast withdrawal from the cell cycle. Because we have previously shown that MyoD is upregulated in FSHD muscle, we wished to determine whether myostatin is also upregulated. Levels of myostatin mRNA are proportional to MyoD levels in myoblasts. At the protein level, myostatin is expressed in myoblasts preparing to fuse, and most robustly in multinucleate cells (myotubes) undergoing nuclear fusion. As might be expected, levels of myostatin, MyoD, and p21 are much lower in myoblasts at low confluence. Thus far we have not observed differences in relative proportions of these factors between FSHD and control myoblasts; however absolute differences secondary to increased MyoD upregulation may contribute significantly to the overall pathogenesis of FSHD. This work was supported by funds from NIAMS R21 and MDA (USA).

**The CAG-repeat polymorphism of the *POLG1* gene is not associated with male oligozoospermia.** C. Michielotto<sup>1,2</sup>, L. Stuppia<sup>3,4</sup>, C. Foresta<sup>5</sup>, M. Zeviani<sup>6</sup>, F. Ferrari<sup>6</sup>, E. Dragone<sup>1</sup>, V. Gatta<sup>3</sup>, S. Pintor<sup>3</sup>, M. Rossato<sup>5</sup>, G. Matullo<sup>2,7</sup>, N. Migone<sup>1,2</sup>, A. Brusco<sup>1,2</sup>. 1) S.C. Genetica Medica, Azienda Osp. San Giovanni Battista, Torino, Italy; 2) Dept. Genetica Biol. Bioch.-Univ. Torino, Italy; 3) Dept. Scienze Biomediche, Univ. Chieti, Italy; 4) ITOI-CNR, Bologna, Italy; 5) Dept. Scienze Mediche e Chirurgiche, Univ. Padova, Italy; 6) Ist. Naz.le Neurologico C.Besta-IRCCS, Milano, Italy; 7) I.S.I, Villa Gualino, Torino.

*POLG1* is a nuclear gene encoding for the catalytic subunit of human mitochondrial DNA polymerase. Absence of the common 10-CAG-repeat allele in *POLG1* has recently been reported in association with a reduced number of spermatozoa (oligozoospermia). To verify these data we studied three case/control cohorts from three different Italian geographical areas. Criteria for inclusion of patients were: (1) moderate oligozoospermia (number of spermatozoa 5-20x10<sup>6</sup>/ml); (2) normal karyotype; (3) absence of Y chromosome deletions in the AZF-a, -b and -c regions. 105 probands from Piedmont (North-Western Italy), 95 from Veneto (North-Eastern Italy), and 77 from Abruzzo (Central Italy), and three sets of controls from each geographical area were selected (Piedmont, N=131; Veneto, N=96; Abruzzo, N=121). Analysis of the *POLG1* gene CAG-repeats was performed through a fluorescent PCR. Within each area, frequencies of the 10/10, 10/X and X/X genotypes did not significantly differ in infertiles vs. controls (P=0.38, 0.19, and 0.32 in Piedmont, Veneto and Abruzzo respectively). The data from the three areas were then pooled, and again the distribution of the three genotypes between infertiles and controls did not show any statistically significant difference (P=0.11). Indeed, the oligozoospermics from all three areas showed a lower frequency of the X/X genotype than in controls (1.9 vs. 5.3%, 1.1 vs. 2.1%, 1.3 vs. 5.0%, and 1.4 vs.4.0% in Piedmont, Veneto, Abruzzo, and in the pool). Our findings suggest that, at least in Italy, the *POLG1* CAG-repeat polymorphisms do not contribute to male oligozoospermia.

**SLUG (SNAI2) is not a common cause of Waardenburg syndrome type 2 (WS2).** *T.A. Maher<sup>1</sup>, C.T. Baldwin<sup>1,2</sup>, G. Zhao<sup>1</sup>, A. Milunsky<sup>1,2</sup>, J.M. Milunsky<sup>1,2,3</sup>.* 1) Center for Human Genetics; 2) Department of Pediatrics; 3) Department of Genetics and Genomics; Boston University School of Medicine, Boston, MA.

Waardenburg syndrome (MIM193500) has an incidence of at least 1 in 40,000 and is one of the most common autosomal dominant syndromes associated with hearing loss. WS is characterized by sensorineural hearing loss, pigmentary abnormalities of the iris, hair, and skin, and is responsible for about 3% of congenital hearing loss. WS is a variably penetrant autosomal dominant disorder that is characterized as a neurocristopathy. Several different types of WS exist, each with different associations. Dystopia canthorum is lacking in WS2, differentiating it from WS1. Using the W-index to distinguish types 1 and 2, our cohort of patients were characterized as WS2. WS2 is a heterogeneous disorder showing linkage to different genomic regions. Approximately 10-20% of WS2 families have mutations in the *MITF* gene. Sequencing of the *MITF* coding sequence revealed 2 mutations (Ex 6 89670 CT ; W241X) in our cohort of 36 unrelated WS2 patients. Recently, 2 unrelated families were reported with a homozygous gene deletion of SLUG (SNAI2 on chromosome 8q11). We examined our cohort of WS2 patients for SLUG gene mutations by sequencing of the coding exons and by southern blot to detect partial or whole gene deletions. No homozygous deletions were found. Sequencing of the coding regions did not detect any mutations. Hence, we conclude that SLUG mutations are not a common cause of WS2.

**Locus CFM1: a potential modifier of pancreatic disease in children with cystic fibrosis.** *M.K. Sontag<sup>1,2</sup>, J.E. Hokanson<sup>2</sup>, J.A. Marshall<sup>2</sup>, M. Corey<sup>3</sup>, G.O. Zerbe<sup>2</sup>, L-C. Tsui<sup>3,4</sup>, J. Zielenski<sup>3</sup>, F.J. Accurso<sup>1</sup>.* 1) Pediatrics, University of Colorado Health Sciences Center, Denver, CO; 2) Preventive Medicine & Biometrics, University of Colorado Health Sciences Center, Denver, CO; 3) Hospital for Sick Children, Toronto, ON, CAN; 4) University of Hong Kong, Hong Kong.

Cystic fibrosis (CF) is a common mendelian disease, with variable phenotype. Immunoreactive trypsinogen (IRT), a pancreatic enzyme precursor, is a marker of pancreatic injury, a significant source of morbidity in CF. IRT is elevated in young children with CF and declines with age, corresponding to pancreatic damage and destruction. Infants with CF who present with Meconium Ileus (MI) have lower IRT levels than infants without MI, suggesting a relationship between pancreatic damage and MI. The CFM1 region on 19q13 is associated with MI in children with CF. To determine if the CFM1 locus is associated with IRT levels we studied serial IRT levels in 288 infants with CF. Rapid, moderate, and slow decliners were identified using a mixed effects model analysis. Genotyping in the CFM1 region is complete on 37 children with CF and both parents. Each trio was genotyped for 6 markers in the CFM1 region. Each of the markers is multi-allelic, and we tested for linkage distortion at each allele. The most significant allele for each marker is presented. Transmission disequilibrium testing (TDT) was performed on rapid decliners (n=9).

Mrkr/allele	D19S408/5	D19S217/4	LOC284248/7	APOC/1	D19S219/1	D19S112/3
<sup>2</sup> (p)	2.0(0.16)	3.6(0.05)	1.0(0.32)	2.0(0.16)	1.3(0.26)	3.0(0.08)

The markers in order (q13.2)(centel),<sup>2</sup>, and p value are shown. The positive association found with allele 4 at marker D19S217 supports other findings that this allele is associated with MI. More families will be screened in order to verify this trend and to look for candidate gene(s) in the CFM1 locus. We conclude that CFM1 is a potential modifier of early pancreatic injury in CF.

**Testing the Position-Effect Variegation Hypothesis for Facioscapulohumeral Muscular Dystrophy By Analysis of Histone Modification and Gene Expression in Subtelomeric 4q.** *M. Ehrlich<sup>1</sup>, G. Jiang<sup>1</sup>, F. Yang<sup>1</sup>, V.*

*Vedanarayanan<sup>2</sup>, P.G. van Overveld<sup>3</sup>, S. van der Maarel<sup>3</sup>.* 1) Human Genetics, Tulane Med. Sch., New Orleans, LA; 2) Dept. of Neurology, Univ. of Mississippi Med. Sch., Jackson, MS; 3) Dept. of Human Genetics, Univ. Leiden, Leiden, The Netherlands.

Facioscapulohumeral muscular dystrophy (FSHD) is a unique dominant disorder involving shortening an array of tandem repeats. This copy-number polymorphic 3.3-kb repeat, D4Z4, is on 4q35 and 10q26 but only 4q35 arrays with <11 copies are linked to FSHD. The most popular model for how the array-shortening causes FSHD is that it leads to D4Z4 heterochromatinization, which spreads proximally along 4q35, causing overexpression of FSHD genes in cis, similar to a loss of position-effect variegation. To test for heterochromatinization in 4q35 subregions, we did chromatin immunoprecipitation (ChIP) analysis of histone H4 acetylation using real-time PCR. First, we showed that there was an average of 2.5-fold less immunoprecipitation (SD, 0.5; N, 14) of constitutive heterochromatin standards than of unexpressed euchromatin standards. Then, in contradiction to the above model for FSHD, we found that H4 acetylation of a non-repeated DNA region adjacent to 4q35 and 10q26 D4Z4 arrays was in the range of unexpressed euchromatin and not of constitutive heterochromatin in both normal and FSHD lymphoid cells. Although ChIP could not be done on muscle, another prediction of the above model was tested by quantitating transcripts from FSHD candidate genes FRG1 and ANT1 on 4q35. Contrary to a recent report, there was no increase in their transcript levels in FSHD skeletal muscle vs. controls. Also, no difference was seen between normal and FSHD lymphoid cells in H4 acetylation at FRG1 5' and ANT1 promoter regions; all were hyperacetylated like expressed gene standards. Therefore, our results favor a different model for the molecular genetic etiology of FSHD, such as, differential long-distance cis looping that depends upon the presence of a 4q35 D4Z4 array with less than a threshold number of copies of the 3.3-kb repeat. Supported in part by FSH Society Grant FSHS-MB-06 and NIH Grant R21 AR48315.

**Mutation of *SFTPC* in infantile pulmonary alveolar proteinosis with or without fibrosing lung disease. M.**

*Bahuau*<sup>1</sup>, *M. Griese*<sup>2</sup>, *S. Marque*<sup>1</sup>, *F. Brasch*<sup>3</sup>, *S. Schumacher*<sup>2</sup>, *J. de Blic*<sup>1</sup>, *C. Houdayer*<sup>1</sup>, *J. Elion*<sup>1</sup>, *R. Couderc*<sup>1</sup>, *M. Tredano*<sup>1</sup>. 1) Biochimie Biologie Molculaire, Hpital Trousseau (MB, SM, CH, RC, MT), Pneumologie, Allergologie Pdiatriques, Hpital des Enfants-Malades (JB), Fdration de Gntique, INSERM U458, Hpital Robert-Debr (JE), AP-HP, Paris, France; 2) Kinderklinik und Kinderpoliklinik, Dr. von Haunerschen Kinderspital, Ludwig-Maximilians Universitt, Mnchen, FRG; 3) Dept. of Pathology, Bergmannsheil, University of Bochum, FRG.

Pulmonary surfactant protein C (SP-C) is a highly hydrophobic peptide produced by type-II alveolar cells by processing of a precursor protein (pro-SP-C), that enhances surface tension and, possibly, facilitates the recycling of pulmonary surfactant. Recently, two dominant-negative mutations of the pro-SP-C-encoding gene (*SFTPC*, MIM 178620), were reported in families with vertically-inherited interstitial lung disease. We have examined the SP-C protein and precursor as well as their encoding gene in 34 sporadic or familial, neonatal and juvenile, cases with unexplained respiratory distress (URD) (SP-B deficiency related to *SFTPB* ruled out). One patient with complete SP-C deficiency had no detectable mutation of *SFTPC*. Of the 10 patients with abnormal pro-SP-C processing, from analysis of broncho-alveolar lavage fluid, two distinct heterozygous *SFTPC* mis-sense mutations were evidenced. The first, g.1286T>C (I73T), inherited de novo, resulted in progressive respiratory failure with intra-alveolar storage of a protein-rich, PAS-positive, material (PAP), and interstitial lung disease. The second, g.2125G>A (R167Q), was found in two PAP patients from the endogamic White settler population of Runion Island in which URD has an unexpectedly high prevalence. Since this mutation was diagnosed in subjects from this subpopulation but without evidence for lung disease, interference of exposures or modifier genes may be proposed. These observations extend the phenotypic spectrum related to *SFTPC* mutation from interstitial lung disease to PAP. Notably, the reported mutations do not appear to be dominant negatives. In-vitro studies are pending to monitor the biosynthesis of the two mutant proteins.

**Primary Ciliary Dyskinesia / Kartagener syndrome: Searching for genes in a highly heterogeneous disorder.** *J.L. Blouin<sup>1</sup>, C. Albrecht<sup>1</sup>, C. Gehrig<sup>1</sup>, G. Duriaux Sail<sup>1</sup>, J.F. Strauss III<sup>2</sup>, L. Bartoloni<sup>1</sup>, C.D. DeLozier Blanchet<sup>1</sup>, S.E. Antonarakis<sup>1</sup>.* 1) Medical Genetics, University Hospitals and School of Medicine, Geneva, Switzerland; 2) University of Pennsylvania, Philadelphia, PA.

Primary Ciliary Dyskinesia (PCD) is an autosomal-recessive disorder with an incidence of 1/20000, characterized by dysmotility of cilium and sperm tail. When respiratory infections and bronchiectasis are associated with situs inversus (50%) the disease is referred to as Kartagener Syndrome (KS). We had shown earlier by linkage analysis an extensive locus heterogeneity in concordance with wide spectrum of axonemal ultrastructural defects. In addition to the loci colocalizing with genes coding for proteins of the dynein arms, defective in 50% of cases, we proposed additional loci (on chromosomes 4q, 19q, 10p, 13q, 15q), which may anchor other PCD genes. To date the genetic causes of PCD/KS have been identified in 10-24% of patients; pathogenic mutations were found in the genes coding for dyneins DNAI1, DNAH5, and DNAH11. In our effort to determine the full spectrum of PCD genes we had screened patients for mutations of other genes DNAH9, HFH4, DNAI2, DNAH3, HP28, DNAL4, DNAL1. In DNAH3, a heterozygous missense substitution (P1197L) was observed in a conserved protein residue, not found in a control population supporting for a pathogenic role of this mutation in PCD. The pathological roles of other missense variants in DNAH9 (P333L), and DNAH3 (A3529D) confirmed as rare polymorphisms remain to be elucidated. We report here a mutation scan in unrelated patients with PCD for three additional reasonable candidate genes. The Sperm Associated Antigen 6 (chr.10p12.2), the human homolog of chlamydomonas PF16 associated with flagellar paralysis, and the Polymerase Lambda (chr.10q24) disrupted in a KO mouse with hydrocephalus, situs inversus and infertility were analysed in a cohort of 54 unrelated patients. The gene for outer arm Beta Heavy Chain DNAH17 was screened in 4 families linked to chr.17q25 where this gene maps. To date, no pathogenic mutations have been found, excluding these genes as major factors of PCD. (Supported by the Swiss National Foundation and the Carvajal foundation of Geneva).

**Analysis of a Fibrillin 1 Pseudoexon and Its Effects on Nonsense Mediated Degradation.** *P.A. Gupta, D.D. Wallis, D. Guo, T. Guidry, V. Tran-Fadulu, D.M. Milewicz.* Dept Internal Medicine, Univ Texas Houston Med Sch, Houston, TX.

Marfan Syndrome (MFS) is an autosomal dominant disorder of the connective tissue. MFS results from mutations in the fibrillin-1 (FBN1) gene. Most mutations are missense mutations, however, there is a significant percentage of mutations that lead to splicing errors. Researchers have predicted that splicing errors might account for ~15% of disease causing mutations. An overwhelming number of these mutations found that lead to alternative splicing result from mutations in the donor or acceptor splice sites, or the branch point, all part of the cis elements that mediate RNA splicing. Conversely, very few reports of pseudoexons have been described. This is the first report to describe the presence of a pseudoexon in FBN1, leading to a milder case of MFS. The pseudoexon results from a creation of a donor splice site in intron 64, leading to the in frame inclusion of a 93 bp fragment of intronic sequence in between exons 63 and 64. The first codon of the pseudoexon is a stop codon. We performed studies that would determine if the pseudoexon with the stop codon initiates nonsense mediated degradation (NMD), a pathway that degrades mRNAs with a premature stop codon (PTC). We believe that NMD reduces the number of mutant alleles, thus reducing the severity of MFS in this family.

**The introduction of exonic splice enhancer elements improves exon inclusion in familial dysautonomia.** *M.M Hims*<sup>1, 2</sup>, *M. Leyne*<sup>1, 2</sup>, *E. Ibrahim*<sup>2</sup>, *J. Mull*<sup>1, 2</sup>, *S.P. Gill*<sup>1, 2</sup>, *R. Reed*<sup>2</sup>, *S.A. Slaugenhaupt*<sup>1, 2</sup>. 1) Massachusetts General Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA.

Familial Dysautonomia (FD) is a progressive degenerative disorder of the sensory and autonomic nervous system with a remarkably high carrier frequency of 1 in 30 in the Ashkenazi Jewish (AJ) population. The major FD mutation is a single-base change at base pair 6 of intron 20 (IVS20+6 T>C) in the *IKBKAP* gene. This mutation results in an apparent decrease in splicing efficiency that leads to variable, tissue-specific skipping of exon 20. We have successfully developed an in vivo splicing minigene system that accurately models the aberrant splicing seen in FD. We have generated mammalian expression vector constructs containing *IKBKAP* exon19-ivs19-exon20-ivs20-exon21 for both the wild-type and FD pre-mRNA sequences. These constructs were transfected into HEK 293T cells, RNA extracted and RT-PCR was carried out to determine which spliced species were produced from the minigenes. Sequence analysis of these RT-PCR products reveals that the majority of spliced transcripts from the FD construct are missing exon 20, while the major spliced transcript from the wild-type construct includes exon 20. These results demonstrate that, in vivo, the FD mutation is sufficient to cause increased skipping of exon 20. Examination of the sequence of *IKBKAP* exons shows that exon 20 has weak splice sites and that exon 20 contains few predicted exonic splice enhancer (ESE) elements. We postulate that the low ESE content and weak splice sites of exon 20 are exacerbated by the IVS20+6 T>C mutation which then leads to deleterious exon skipping. Furthermore, we have shown that the introduction of known ESE sequences into exon 20 of the *IKBKAP* minigenes by site directed mutagenesis, can improve the efficiency of exon 20 inclusion and can compensate for the negative effects of the IVS20+6 T>C mutation. These results suggest that the tissue specific exon skipping seen in FD patients may be related to the role of ESEs in enhancing exon 20 inclusion in specific tissues, and that our studies will yield valuable insight into the mechanism of tissue-specific splicing.

**The myotonic dystrophy type 2 (DM2) CCTG expansion does not prevent allele specific *ZNF9* expression.** *J.M. Malikowski<sup>1</sup>, M.L. Moseley<sup>1</sup>, A.K. Mosemiller<sup>1</sup>, D. Jin<sup>1</sup>, B. Schooser<sup>2</sup>, W. Kress<sup>3</sup>, K. Ricker<sup>3</sup>, J.W. Day<sup>1</sup>, L.P.W. Ranum<sup>1</sup>.*  
1) Institute of Human Genetics, University of Minnesota, Minneapolis, MN; 2) Ludwig-Maximilians-University, Germany; 3) University of Wurzburg, Germany.

We recently demonstrated that myotonic dystrophy type 2 is caused by a CCTG expansion in intron 1 of the *zinc finger protein 9 (ZNF9)* gene. Similar to DM1, RNA foci containing the repeat accumulate in nuclei of affected DM2 tissue. DM2 expansions (75-11,000 CCTGs, mean ~20kb) are often much larger than for other expansion disorders. To understand the molecular consequences of the DM2 expansion we performed a series of experiments with haploid and homozygous cell lines to determine the effects of the DM2 expansion on *ZNF9* expression. RT-PCR on chromosome separated mouse-human hybrid cell lines containing a single copy of the normal or expanded (1,000 CCTGs) human DM2 allele indicates that the CCTG expansion does not prevent allele specific splicing. Consistent with normal splicing, RNA in situ hybridization in control and expansion carriers indicates that exon 1 and exon 5 (5' and 3' of the CCTG, respectively) localize primarily to the cytoplasm but not to the CCUG-containing nuclear foci. The identification of a rare patient homozygous for the DM2 expansion has provided an opportunity to examine the effects of very large CCTG expansions (>5000 CCTGs) on *ZNF9* expression without the complications introduced by the expression of the murine homolog or the normal human allele. Northern analysis demonstrates that *ZNF9* is expressed at comparable levels in myoblasts generated from control as well as DM2 patients heterozygous or homozygous for the CCTG expansion. Western analyses of myoblast cell lines using polyclonal antibodies generated against *ZNF9* show comparable levels of *ZNF9* protein in myoblast cell lines from control, heterozygous and homozygous expansion carriers. In summary, even very large DM2 expansions (>5000 CCTGs) do not prevent transcription or splicing of *ZNF9* and do not appear to affect overall *ZNF9* protein levels in homozygous myoblasts. These results are consistent with a model in which CCUG expansions themselves alter cellular function causing DM2.

**Mutations at the RMRP (Cartilage-Hair Hypoplasia) Gene: Haplotyping and Comparative Genomics.** *L. Bonafe*<sup>1</sup>, *E.T. Dermitzakis*<sup>2</sup>, *S.E. Antonarakis*<sup>2</sup>, *A. Superti-Furga*<sup>1</sup>, *A. Reymond*<sup>2</sup>. 1) Division of Molecular Pediatrics, University of Lausanne, Switzerland; 2) Division of Medical Genetics, University of Geneva, Switzerland.

The RMRP gene on chromosome 9 has several unusual features. It is small, with a promoter region of approx. 300 bp and a transcribed intronless region of 267 bp; and its transcript remains untranslated as it associates as an RNA component to the RNase MRP complex. In spite of its small size, we have identified no less than eight SNPs (four within its promoter, three in the transcribed region, one immediately 3'), and we and others have identified over forty different putative pathogenic mutations in individuals with Cartilage-Hair Hypoplasia variants. The putative pathogenic mutations can be divided into two classes: small insertions (6 to 30 bp) around the 5' of the transcribed region; and substitutions, insertions and deletions of single or adjacent nucleotides within the transcribed region. Because no functional assay for RMRP activity is available, and there seem not to be clear genotype-phenotype correlations, assessment of the sequence changes as pathogenic or neutral polymorphisms relies only on segregation within affected families and/or presence in randomly chosen control individuals. To address the significance of sequence changes identified in humans, we compared the sequence of human RMRP against a set of orthologous sequences covering the entire Eutherian tree and an outgroup from the Metatheria (human, monkey, lemur, mouse, porcupine, rabbit, pig, cat, shrew, armadillo, elephant and wallaby). Most putative pathogenic mutations in the transcribed region (27 positions out of 31), but not the polymorphisms (0 position out of 3), affect nucleotides that are strongly conserved. The eight SNPs show linkage disequilibrium and define two main groups that differ by 4 SNPs. No obvious ethnic association could be identified. Both the frequency and the spectrum of sequence changes identified at the RMRP gene are unusual; it is unclear whether this is caused by specific mutational mechanisms and/or by a different selective pressure.

**Functional significance of the carboxy-terminus of the Wilson disease copper-transporting ATPase.** *G. Hsi, L.M. Cullen, D.M. Glerum, D.W. Cox.* Medical Genetics, Univ Alberta, Edmonton, AB, Canada.

Wilson disease (WND) is a disorder of copper transport in which copper accumulates in the liver, brain and kidneys. The defective gene, *ATP7B*, encodes a copper-transporting ATPase, ATP7B. We investigated the significance of the carboxy (C)-terminus of ATP7B with respect to its biosynthetic role in copper delivery to the ferroxidase ceruloplasmin. The C-terminal region has not been extensively analyzed. Its importance for function is unclear as there is not a high prevalence of disease-causing mutations in the C-terminus of ATP7B, with only two missense mutations and one deletion reported in patients. This suggests that either there are only a few critical amino acids in the region necessary for function or sequence changes cause mild disease that remain undiagnosed. We used an assay that exploits the similarities between the yeast and human copper transport systems to assess the functional capabilities of ATP7B variants. The yeast ferroxidase, Fet3p, requires copper from the yeast homologue of ATP7B, Ccc2p, and cannot function in the presence of a nonfunctional Ccc2p; expression of wild-type human *ATP7B* in *ccc2* mutant yeast complements the iron-deficient phenotype. We demonstrated that the C-terminus of ATP7B appears to be necessary for protein stability, as removal of the entire non-membrane terminus leads to reduced protein production and cessation of growth in iron-limited media. However, growth is partially restored when an additional three amino acids are present, and is near wild-type levels when two-thirds of the C-terminus is removed. Measurement of ferroxidase activity is a more sensitive indicator of copper transport function: a deletion of two-thirds of the C-terminus of ATP7B resulted in slight impairment in the ability to deliver copper to Fet3p. Two patient missense mutations exhibited full complementation of the yeast *ccc2* phenotype and thus are not impaired in their ability to transport copper to Fet3p. However, these mutations may still affect ATP7B function as the protein has an additional role within the hepatocyte involving copper trafficking prior to biliary excretion. This aspect can be assessed further in a mammalian cell assay.

**Characterisation of two novel splicing enhancer sequences in the dystrophin gene, located within a LINE1**

**element and involved in XLDC.** *P. Rimessi<sup>1</sup>, F. Gualandi<sup>1</sup>, T. Patarnello<sup>2</sup>, F. Muntoni<sup>3</sup>, E. Calzolari<sup>1</sup>, G.*

*D'Agostaro<sup>1</sup>, A. Ferlini<sup>1</sup>.* 1) Section of Medical Genetics, University of Ferrara, Ferrara, Italy; 2) Dipartimento di Biologia, University of Padova (Italy); 3) Department of Paediatrics, Hammersmith Hospital Campus, Imperial College, London (UK).

Splicing mutations are responsible for the 15% of human genetic diseases and are often regulated in a tissue/cell-specific manner. A relevant category of sequences required for both constitutive and alternative splicing are splicing enhancers. These RNA motifs are located within exons or introns and participate to the exon definition process. SR proteins, a family of essential splicing factors, are recruited directly to the RNA by the enhancer elements or indirectly by interactions with other general splicing factors. Mobile elements, accounting for at least 40% of the human introns, include LINE and Alu sequences which are known to generate protein diversity by using different mechanisms as 3 transduction and exon shuffling. We identified and functionally characterised two purine-rich regions located within dystrophin intron 11 and involved in splicing regulation. The role of these motifs was highlighted by an intronic dystrophin mutation causing X-linked dilated cardiomyopathy (XLDC) and determining the tissue-specific incorporation of an aberrant exon into the dystrophin transcript. These two motifs, as well as the 5 cryptic splice site used by the aberrant exon in the XLDC family, are contained within an atypical L1 5 region. The first splicing sequence occurs within the aberrant XLDC exon while the second one is localised within its 3 intron. These sequences behave in vitro as splicing enhancers because their deletion strongly interferes with the aberrant dystrophin exon inclusion. Our results suggest that these motifs might be involved in the pathogenesis of the XLDC in this family. RNA electrophoresis mobility shift assays (REMSA) demonstrate that the enhancer sequences interact with proteins in HeLa nuclear extracts. We are currently identifying the specific SR protein(s) binding the splicing motifs identified. Acknowledgement The EU FINGER and the Telethon-Italy grants are acknowledged.

**Spinocerebellar ataxia type 10 ATTCT pentanucleotide repeat expansions in multiple Brazilian families.** *P.*

*Fang<sup>1</sup>, H.A.G. Teive<sup>2</sup>, I. Alonso<sup>3</sup>, L. Jardim<sup>4</sup>, J. Sequeiros<sup>3</sup>, I. Silveira<sup>3</sup>, S. Raskin<sup>5</sup>, E. Schmitt<sup>1</sup>, P.A. Ward<sup>1</sup>, T. Matsuura<sup>1</sup>, T. Ashizawa<sup>6</sup>, B.B. Roa<sup>1</sup>.* 1) Dept Molec Human Genetics, Baylor Col Medicine, Houston, TX; 2) Dept Medicine, Federal Univ Parana, Brazil; 3) UnIGENE, IBMC, Univ of Porto, Portugal; 4) Dept Medical Genetics, Hospital de Clinicas, Porto Alegre, Brazil; 5) Laboratorio Genetika, Brazil; 6) Dept Neurology, The University of Texas Medical Branch, Galveston, TX.

Spinocerebellar ataxia type 10 (SCA10) is an autosomal dominant neurogenetic disorder characterized by cerebellar ataxia and seizures. SCA10 is associated with a non-coding ATTCT pentanucleotide repeat expansion in the SCA10 gene, with a normal range of 10-22 repeats, and expansion mutations ranging in the hundreds to thousands of repeats. SCA10 was previously reported primarily in ataxia patients of Mexican ancestry. Our molecular studies have identified SCA10 expansions in a total of eight unrelated families of Brazilian ancestry. Affected individuals from six unrelated Brazilian kindreds were referred for clinical testing at the Baylor DNA Diagnostic laboratory. SCA10 expansion mutations were identified in these SCA10 patients, ranging from ~1600 to ~2300 repeats determined by Southern analysis. The clinical findings in these patients include progressive gait ataxia, abnormal cerebellar signs and nystagmus. Seizures were not observed in these Brazilian patients, in contrast to previous reports on Mexican SCA10 patients. One Brazilian family was documented to exhibit anticipation through maternal transmission, wherein the mother had an expanded allele of ~1600 repeats and a reported age of onset ~40 years, while her affected child had ~1900 repeats and an earlier onset at ~30 years of age. In addition, two other Brazilian families tested positive for SCA10 expansions using modified PCR-based methods. Patients from these families also showed symptoms of progressive cerebellar ataxia without seizures. Our collective molecular studies on hundreds of ataxia patients in North America and Portugal indicate that SCA10 is a rare form of hereditary ataxia, with a significant incidence that appears to be limited to the Mexican and Brazilian populations.

**Mosaicism for *FMR1* gene full mutation and deletion in a fragile X female.** *H. Fan*<sup>1</sup>, *J.K. Booker*<sup>1</sup>, *S.E. McCandless*<sup>1</sup>, *V. Shashi*<sup>2</sup>, *R.A. Farber*<sup>1</sup>. 1) University of North Carolina, Chapel Hill, NC; 2) Wake Forest University School of Medicine, Winston-Salem, NC.

Most cases of fragile X syndrome result from expansion of CGG repeats in the *FMR1* gene; deletions and point mutations of *FMR1* are much less common. Mosaicism of an *FMR1* full mutation with a deletion or with a normal allele has been reported in fragile X males. Here we report on a fragile X female who is mosaic for an *FMR1* full mutation and an intragenic deletion. The patient is a 3-year-old girl with developmental delay, autistic-like behaviors and significant speech and language abnormalities. The *FMR1* trinucleotide repeat mutation was analyzed by Southern blotting of peripheral blood DNA digested with EcoR I and Eag I, using a <sup>32</sup>P-labeled StB 12.3 probe. The autoradiograph demonstrated the presence of a methylated full mutation at 6.4 kb (expansion to about 440 repeats), a normal female allele in methylated (5.2 kb) and unmethylated (2.8 kb) forms, and an additional fragment at 5.0 kb. This result indicates that the patient is mosaic for a full mutation and a deletion, in the presence of a normal allele. The extent of the deletion was determined by PCR amplification and sequencing of the DNA flanking the breakpoint. As expected, the normal allele and deletion allele amplified, but the expanded allele did not. We mapped the 5' breakpoint 63/65 bp upstream from the CGG repeat region and the 3' breakpoint 86/88 bp downstream of the CGG repeats within the *FMR1* gene. (The exact breakpoints cannot be determined because of the presence of a GG sequence at both ends of the deletion.) The deletion removed 210 bp, including the entire CGG repeat region. The full mutation was presumably inherited from a premutation or full mutation in the patient's mother (studies pending). The deletion, which remained methylated at the Eag I site, was probably derived from the full mutation allele. Mosaicism of this type is rare in females with a fragile X mutation, but should be kept in mind in interpretation of Southern blots. In this case, the deletion does not appear to affect the phenotype of the patient.

**Polyglutamine expansions induce irreversible chromatin remodelling and alteration of photoreceptors maintenance in SCA7 and HD transgenic mice.** *D. Devys<sup>1</sup>, D. Helmlinger<sup>1</sup>, G. Abou-Sleymane<sup>1</sup>, G. Yvert<sup>1</sup>, Y. Trottier<sup>1</sup>, S. Picaud<sup>2</sup>, J.L. Mandel<sup>1</sup>.* 1) Molecular Pathology, IGBMC, Illkirch, France; 2) INSERM EMI-99-18, Paris, France.

Spinocerebellar ataxia type 7 (SCA7) and Huntingtons disease (HD) belong to a group of nine inherited progressive neurodegenerative diseases caused by a CAG/polyglutamine (polyQ) expansion and characterized by continuous accumulation of mutant protein. Although retinal degeneration is only observed in SCA7, we described a retinal phenotype in an HD mouse model comparable to that found in our SCA7 transgenic mice. In SCA7 mice, mutant ataxin-7 is expressed exclusively in photoreceptors and induces a progressive and long-lasting retinal dysfunction but a delayed and limited cell loss, even in 2.5 years old mice. We also observed that rod photoreceptor chromatin structure was dramatically altered leading to a strong downregulation of rhodopsin and other genes coding for proteins of the phototransduction pathway. Mutant ataxin-7 transgene which expression is driven by rhodopsin promoter was also early and dramatically downregulated. Surprisingly, mutant ataxin-7 extinction contrasted with a constant worsening of the retinal phenotype leading to a complete loss of photoreceptor activity. A previous study reported that abolishing mutant huntingtin expression reversed neurological phenotype in an inducible HD mouse model. We demonstrate here that retinopathy progression in SCA7 mice is not dependent upon continuous expression of mutant ataxin-7, raising the possibility that polyQ induced neuronal dysfunction may be irreversible.

**Polyglutamine aggregation impairs nuclear export of huntingtin.** *J.C. Cornett<sup>1, 2</sup>, S.H. Li<sup>1</sup>, X.J. Li<sup>1</sup>*. 1) Dept Human Genetics, Emory University, Atlanta, GA; 2) Graduate Program in Genetics and Molecular Biology.

Huntingtons disease (HD) is one of at least nine distinct neurological diseases caused by expanded polyglutamine repeats in the disease proteins. Nuclear accumulation and aggregation of polyglutamine proteins are common features of these diseases, suggesting a close link between nuclear localization and toxicity. In HD, N-terminal huntingtin fragments accumulate in the nucleus and form inclusions whereas full-length huntingtin is predominantly localized in the cytoplasm. Lack of conserved nuclear import or export sequences in N-terminal htt suggests that small N-terminal huntingtin may passively shuttle into and out of the nucleus. We treated huntingtin exon 1 stably-transfected HEK293 and PC12 cells with Leptomycin B (LMB), a nuclear export inhibitor. In both cell lines LMB treatment led to an increase in nuclear huntingtin. LMB had no effect on the distribution of full-length huntingtin in stably-transfected HEK293 cells. This suggests that N-terminal huntingtin shuttles into and out of the nucleus such that inhibition of nuclear export can increase its concentration in the nucleus. To investigate whether the nuclear accumulation of N-terminal huntingtin is dependent on Ran GTPase, which is found to be critical for the nuclear export of certain types of RNAs and molecules, we transfected huntingtin exon 1 containing 23Q or 120Q into tsBN2 cells. These cells have a temperature sensitive mutation resulting in the suppression of Ran-dependent nuclear transport at the non-permissive temperature. The nuclear distribution of transfected huntingtin was not altered from the permissive temperature to the non-permissive temperature. Thus, huntingtin's nuclear accumulation is unlikely dependent on Ran. Transfected NLS-tagged huntingtin exon 1 with 120Q was present as soluble and aggregated proteins in the nucleus. When shifted to the non-permissive temperature, the soluble pool of mutant huntingtin became diffuse in the cytoplasm while aggregated huntingtin remained in the nucleus. This result suggests that soluble huntingtin may be exported from the nucleus via an unknown mechanism but polyglutamine aggregation impairs the nuclear export of huntingtin.

**Genetic study of Portuguese patients with a Huntington disease-like phenotype.** *M.C. Costa<sup>1,2</sup>, M. Constante Pereira<sup>1</sup>, P. Magalhães<sup>1</sup>, M.T. Matamá<sup>1</sup>, F. Ferreirinha<sup>1</sup>, J. Cerqueira<sup>1</sup>, M.M. Santos<sup>1,3</sup>, J. Sequeiros<sup>1,4</sup>, P. Maciel<sup>1,2,4</sup>.*  
1) UnIGENE, IBMC, Univ. Porto, Portugal; 2) Life and Health Sciences Research Institute, School of Health Sciences, Univ. Minho, Portugal; 3) Department of Medicine, CHUM, Hôpital Notre-Dame, Univ. Montréal, Canada; 4) Department of Populations Studies, ICBAS, Univ. Porto, Portugal.

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterised by involuntary choreiform movements, cognitive impairment, dementia and personality changes. A few patients are known with a similar clinical presentation but no expansion of the CAG repeat on the *HD* gene (Huntington disease-like, or HD-L). We now report a group of 95 Portuguese patients with a HD-L phenotype, including 47 with typical and 48 with unusual HD symptoms (36 presented only the movement disorder, while 12 had only psychiatric symptoms). Overall mean age at onset was 46.2±9.9 years (range, 3-83). Given that SCA17 and DRPLA share some of the clinical features with HD, their causative genes, encoding for TATA-binding protein (*TBP*) and atrophin-1 (*DRPLA*), were also screened for repeat expansions. These HD-L patients were then screened for repeat expansions on the genes *HDL1* and *HDL2* genes encoding, respectively, for junctophilin-3 and prion protein. In addition, because of some clinical overlap, namely of the movement disorder, between HD and neuroferritinopathy, the adenine insertion (460-461InsA) on the *FTL* gene, which encodes for ferritin light polypeptide, was also screened in all HD-L patients; the entire *FTL* gene was sequenced for those patients presenting only movement incoordination (n=30). Expansions on the repetitive tracts of the *HDL1*, *HDL2*, *TBP* and *DRPLA* genes, as well as the 460-461InsA on the *FTL* gene, were excluded in all patients; also, no other mutations were found on the *FTL* gene for the patients studied. None of the genes that should be included in the differential diagnosis of HD was responsible for the disease on our series. Further investigation on the genetic heterogeneity of HD-L is still needed.

**Intranuclear neuronal inclusions in two female carriers of the fragile X premutation.** *C.M. Greco<sup>1</sup>, F. Tassone<sup>2</sup>, S. Jaquemont<sup>3</sup>, R.J. Hagerman<sup>3</sup>, P.K. Sahota<sup>2</sup>, A. Delacourte<sup>4</sup>, C.A. Maurage<sup>4</sup>, P.J. Hagerman<sup>2</sup>.* 1) Department of Pathology, University of California at Davis, Sacramento, CA; 2) Department of Biological Chemistry, UC Davis, Davis, CA; 3) MIND Institute, University of California Davis Medical Center, Sacramento, CA; 4) Service d'Anatomie et Cytologie Pathologique, CHU, Lille.

Premutation alleles of the fragile X mental retardation 1 (FMR1) gene (55 to 200 CGG repeats) have been found to cause a neurodegenerative disorder, fragile X-associated tremor/ataxia syndrome (FXTAS), in some males over 50 yr. The major clinical features of FXTAS are progressive intention tremor and/or cerebellar gait ataxia, dementia, parkinsonism, autonomic dysfunction, and peripheral neuropathy. Here, we report the first cases of female carriers of the fragile X premutation presenting with neurological symptoms and neuronal intranuclear inclusions. Case 1 (80 CGG repeats, AR 0.3) experienced her first symptoms at the age of 56 years with expressive and receptive aphasia. She subsequently developed hyperphagia, incontinence, progressive frontotemporal symptoms, hallucinations, and swallowing difficulties. She expired at 66 years. Neurohistological analysis showed ubiquitin positive intraneuronal and astrocytic inclusions, similar to those previously described in male carriers of the fragile X premutation. Additionally, histologic features diagnostic of Alzheimers disease were present. Case 2 (87 CGG repeats, AR 0.6) had mild tremor for the last 3 years and ataxia for the last year prior to her death. She did not have any significant cognitive deficit (FSIQ was 100). Preliminary results of histologic examination of the brain show intranuclear inclusions in neurons and astrocytes without other significant pathology. We also analyzed brain tissue from an asymptomatic female premutation carrier who died at the age of 76 of uterine cancer. Brain gross examination and neurohistological analysis did not show any abnormalities. Her CGG repeats were 30, 70 with an AR of 0.8. These findings suggest that FXTAS is a neurodegenerative syndrome that can be observed not only in premutation males but also rarely in female premutation carriers.

**Expanded Ataxin-3 Enhances Staurosporine-Induced Neuronal Cell Death and Decreases bcl-2 Expression.** *M. Hsieh*<sup>1,2</sup>, *H.-F. Tsai*<sup>3,4</sup>, *S.-Y. Li*<sup>5</sup>. 1) Dept Life Sci, TungHai Univ, Taichung, Taiwan; 2) Life Science Research Center, TungHai Univ, Taichung, Taiwan; 3) Institute of Medicine, Chung Shan Med Univ, Taichung, Taiwan; 4) School of Medical Technology, Chung Shan Med Univ, Taichung, Taiwan; 5) Dept Life Sci, Chung Shan Med Univ, Taichung, Taiwan.

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. Apoptosis was previously shown to lead to neural cell death of the disease. In this study, we utilized human neuroblastoma SK-N-SH cells stably transfected with HA-tagged full-length MJD with 78 polyglutamine repeats to examine the effects of polyglutamine expansion on neuronal cell survival under different apoptotic stress. Various pro-apoptotic agents were used to assess the tolerance of the mutant cells and to compare the differences between cells with and without mutant ataxin-3. Concentration and time course experiments showed that the increase in staurosporine-induced cell death was more pronounced and accelerated in cells containing expanded ataxin-3 via MTS assays. Microscopic observations also demonstrated that cells expressing expanded ataxin-3 were more sensitive to staurosporine-induced apoptosis. Interestingly, under basal conditions, Western blot and immunocytochemical analysis showed a significant decrease of Bcl-2 protein expression in cells containing expanded ataxin-3 when compared with that of the parental cells. However, the protein levels of Bax and Bcl-xL were not significantly altered. These results indicated that expanded ataxin-3 that leads to neurodegenerative disorder significantly impaired the expression of Bcl-2 protein, which may be responsible, at least in part, for the weak tolerance to apoptotic stress and ultimately resulted in an increase stress-induced cell death upon stress.

**Consequences of polyglutamine and polyalanine expansions in the context of the protein Ataxin7.** *M. Latouche<sup>1</sup>, P. Fragner<sup>1</sup>, K.H. El Hachimi<sup>2</sup>, E. Martin<sup>1</sup>, A. Sittler<sup>1</sup>, A. Brice<sup>1</sup>, G. Stevanin<sup>1</sup>.* 1) INSERM-U289-NEB, Paris, France; 2) INSERM U106, Paris, France.

Spinocerebellar ataxia type 7 (SCA7) is caused by polyglutamine (polyQ) expansions in Ataxin7. PolyQ diseases are characterized by the presence of intranuclear inclusions (NIIs) due to the inherent ability of polyQ-mutated proteins to aggregate. To determine the role of the nature of the repeated amino acid vs the protein context and the role of aggregation in the pathological process, we produced and compared cellular models with polyalanine (Ataxin7-90A) and polyQ expansions (Ataxin7-100Q). Ataxin7-90A and Ataxin7-100Q were transiently expressed in HEK293 cells and in primary cultures of rat embryonic mesencephalic neurons. In both cases (Ataxin7-90A and -100Q) the formation of nuclear and perinuclear aggregates was observed. In addition, the presence of chaperones and components of the Ubiquitin-Proteasome system in these NIIs points to an abnormal folding of the expanded proteins. However, the aggregates formed by Ataxin7-90A, as visualized using optic microscopy, are morphologically distinct from the Ataxin7-100Q NIIs. Furthermore, at the ultrastructural level, amorphous deposits of Ataxin7-90A, but no fibrillar inclusions as observed with Ataxin7-100Q, were detected. Finally, Ataxin7-90A was more toxic than Ataxin7-100Q in primary cultures of mesencephalic neurons.

**Expression of expanded polyglutamine protein induces behavioral changes in *Drosophila*.** *S.M. Shin*<sup>1,2</sup>, *Y.T. Kim*<sup>2</sup>, *W.Y. Lee*<sup>3</sup>, *G.M. Kim*<sup>3</sup>, *D.K. Jin*<sup>4</sup>. 1) Clinical Research Center, Samsung Biomedical Research Institute, 50 Ilwon-dong, Kangnam-gu, Seoul 135-710, South Korea; 2) Department of Life Science, Sogang University, 1 Shinsu-dong, Mapo-gu, Seoul 121-742, South Korea; 3) Department of Neurology, Sungkyunkwan University, School of Medicine, Samsung Medical Center, 50- Ilwon-dong, Kangnam-gu, Seoul 135-710, South Korea; 4) Department of Pediatrics, Sungkyunkwan University, School of Medicine, Samsung Medical Center, 50-Ilwon-dong, Kangnam-gu, Seoul 135-710, South Korea.

Spinocerebellar ataxia type-3 or Machado-Joseph disease (SCA3/MJD) is an autosomal dominant neurodegenerative disease caused by triplet nucleotide expansion. The expansion of the polyglutamine tract near the C-terminus of the *MJD1* gene product, ataxin-3, above a threshold of 40 glutamine repeats causes neuronal loss and degeneration. The expanded ataxin-3 forms aggregates, and nuclear inclusions (NIs), within neurons, possibly due to the misfolding of mutant proteins.

Here we report upon the behavioral test changes related to truncated and expanded forms of MJD protein (MJDtr) in *Drosophila*, and show that expanded MJDtr, when expressed in the nervous system causes characteristic locomotor dysfunction and anosmia. This phenomenon has not been previously reported in transgenic *Drosophila* models. In addition, *in vivo* expression of anti-apoptotic gene *bcl-2* showed no evidence of ameliorating the deleterious effect of MJDtr-Q78s, either in the eye or the in the nervous system. The study shows that such transgenic *Drosophila* models express ataxic behavior as observed in human patients.

Program Nr: 2456 from 2003 ASHG Annual Meeting

**The FMR1 premutation and spinocerebellar ataxia.** *K. Storm, F. Kooy, N. Peeters, M. Van den Bergh, L. Vits.*  
Medical Genetics, University of Antwerp, Antwerp, Belgium.

**Background:** Recent studies report the clinical involvement of the fragile X (FMR1) premutation in male carriers affected by a characteristic late-onset syndrome involving progressive action tremor with ataxia, cognitive decline and generalized brain atrophy, also called fragile X-associated tremor/ataxia syndrome or FXTAS. The symptoms of FXTAS may be confused with those of spinocerebellar ataxia. **Objective:** To explore the relative frequency of FMR1 premutations among familial and sporadic spinocerebellar ataxias, a cohort of 97 male patients with neurodegenerative disorders referred for genetic analysis of the spinocerebellar ataxia genes and found to be negative for the SCA1, 2, 3, 6 and 7 mutations were analyzed by PCR amplification / Southern blot to determine the CGG-repeat size in the FMR1 gene. **Results:** FMR1 premutations were not detected in any of the cases investigated so far. The largest allele found contains 48 repeats, a finding within the non-pathogenic range (< 200 CGG-repeats). **Conclusion:** These preliminary results indicate that the FMR1 premutation may only be a rare cause of spinocerebellar degeneration. Additional studies of larger cohorts of SCA-negative patients are required to ascertain the relative frequency of FMR1 premutations and also to determine the boundaries of the CGG-repeat expansion associated with this disorder. Studies may be extended to patients with parkinsonian signs.

Program Nr: 2457 from 2003 ASHG Annual Meeting

**Probable mechanism of trinucleotide repeat expansion in a patient with SCA17.** *A. Shatunov, F. Pagan, E. Fridman, J. Leib, M. Hallett, L. Goldfarb.* National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, US.

Expansion of glutamine coding repeats in at least nine genes causes well defined neurodegenerative disorders, including Huntingtons disease and Spinocerebellar ataxias. Polyglutamine expansion in the TATA-box-binding protein (TBP) has recently been associated with SCA17. We observed a sporadic case of prominent ataxia and progressive intellectual decline in a young American woman with disease onset at age 19. The patient showed 55 triplet repeats in the TBP gene and was diagnosed with SCA17. The patients unaffected parents have normal length alleles. The expansion in the TBP gene differs from other genes by the presence of multiple CAG and CAA triplets organized in recognizable blocks and therefore allowing structural analysis. The patients expanded repeat area is composed of the paternal full-length repeat region in tandem with a downstream part of the paternal second repeat allele. The patients normal allele has a distinct sequence coming from the mother. Our data based on haplotype and sequence analyses of the TBP gene region in the patient and her parents suggest that the patients father is germ-line mosaic, and unequal crossover is a likely mechanism by which the pathogenic allele was generated.

**Intranuclear inclusions in fragile X-associated tremor/ataxia syndrome neuronal cells from five premutation carriers.** *F. Tassone*<sup>1</sup>, *R.J. Hagerman*<sup>2,3</sup>, *D. Garcia*<sup>1</sup>, *P.K. Sahota*<sup>1</sup>, *E.W. Khandjian*<sup>4</sup>, *C.M. Greco*<sup>5</sup>, *P.J. Hagerman*<sup>1</sup>. 1) Dept Biological Chemistry, Univ California, Davis, Davis, CA. Department of Biological chemistry, School of Medicine, UC Davis, Davis, CA; 2) MIND Institute, University of California Davis Medical center, Sacramento, CA; 3) Department of Pediatrics, University of California Davis Medical Center, Sacramento, CA; 4) Department of Medical Biology, Laval University, Quebec, Canada; 5) Department of Pathology, University of California Medical Center, Sacramento, CA.

A neurodegenerative disorder, fragile X-associated tremor/ataxia syndrome (FXTAS) has recently been described in a subgroup of adult male carriers of the fragile X mental retardation 1 (FMR1) premutation (55 to 200 CGG repeats). Clinical involvement is associated with the presence of eosinophilic intranuclear inclusions in neuronal and astroglial cells throughout the brain. To investigate the possibility that larger (full mutation) expansions may be present in the cells that harbor the inclusions in brain (neural mosaicism) we have performed detailed post-mortem neuropathological and molecular analysis of multiple regions throughout the brain of five male carriers of premutation alleles, who had presented with neurological symptoms consistent with FXTAS prior to death. DNA genotype analysis revealed a lack of any inter-region allelic size heterogeneity; premutation alleles of the same CGG repeat number were present in all brain regions examined and were also identical to the allele size present in peripheral blood. Neuropathology studies revealed the presence of intranuclear inclusions in both neurons and astrocytes, and cerebellar neurodegeneration that included Purkinje cell loss and axonal swelling in all five premutation carriers. This study establishes that brain tissues with premutation alleles are subject to a specific, fragile X-associated neuropathology that includes neuronal intranuclear inclusion formation, and excludes the possibility that cryptic, full mutation alleles are responsible for either inclusion formation or FXTAS.

**Small-pool PCR analysis of premutation alleles at the *FRDA* (frataxin) locus.** *R. Sharma*<sup>1</sup>, *M. Gomez*<sup>1</sup>, *S. Shah*<sup>1</sup>, *M. Delatycki*<sup>2</sup>, *L. Pianese*<sup>3</sup>, *A. Monticelli*<sup>4</sup>, *B. Keats*<sup>5</sup>, *S.I. Bidichandani*<sup>1</sup>. 1) Dept Biochem & Molec Biol, Oklahoma Univ Health Sci, Oklahoma City, OK; 2) Murdoch Institute, Australia; 3) BIOGEM, Italy; 4) IEOS CNR-DBPCM, Naples, Italy; 5) Genetics, LSU Medical Center, LA.

Friedreich ataxia is caused by abnormal expansions of a GAA triplet-repeat sequence in intron 1 of the *FRDA* gene. This repeat is highly polymorphic; normal alleles contain 5-30 triplets and disease-causing alleles contain 100-1700 triplet-repeats. Rare alleles with 30-100 triplet-repeats, termed premutations, do not normally cause disease, but may expand into the pathogenic range when transmitted from parent to child. It is not known at what stage hyperexpansion of premutation alleles occurs, i.e., does it occur in mitotic division of pre-germ cells, during meiosis, or in post-fertilization mitotic division? We decided to analyze premutation alleles by SP-PCR of peripheral blood leukocytes in order to investigate if expansion occurs during mitotic division. We analyzed a total of 4581 individual DNA molecules from carriers of seven alleles ranging in size from 30-105 triplet-repeats. While the GAA-30 and GAA-39 alleles were stable, alleles containing 66-105 were somatically unstable. The average mutation load was 12.1% with 8.5% contractions and 3.6% expansions. Our previous demonstration of instability in an allele with 44 triplet-repeats means that the threshold length for the initiation of somatic instability at the *FRDA* locus is between 40 and 44 triplets, which is approximately the size of a eukaryotic Okazaki fragment. Contrary to the expansions seen in parental transmissions, these alleles showed only small slippage type events, with a paradoxical bias towards contractions. In fact, the GAA-39 allele, which was stable in our SP-PCR assay, underwent hyperexpansion in two parent-to-child transmissions. These data demonstrate that the process of hyperexpansion is unlikely to occur during mitotic division of somatic cells. Furthermore, the fact that the threshold length for the initiation of instability corresponds to the size of a eukaryotic Okazaki fragment suggests that somatic instability may stem from errors in lagging strand synthesis.

**Nuclear localization of polyglutamine-expanded Androgen Receptor is not required for Transglutaminase-potentiated proteasome dysfunction.** *T.C. Scanlon<sup>1,2</sup>, L.K. Beitel<sup>1,3</sup>, B. Gottlieb<sup>1</sup>, M.A. Trifiro<sup>1,2,3</sup>.* 1) Lady Davis Institute, Montreal, Quebec, H3T 1E2, Canada; 2) Department of Human Genetics, McGill University, Montreal, Quebec H3A 1B1, Canada; 3) Department of Medicine, McGill University, Montreal, Quebec H3A 1B1, Canada.

The polyglutamine (polyQ) expansion in the Androgen Receptor (AR) has been shown to be the molecular insult responsible for Spinal Bulbar Muscular Atrophy (SBMA). PolyQ-expanded tracts could act as good glutamyl donors in reactions catalyzed by Transglutaminase(TG). TG-mediated isopeptide bonds are proteolytically resistant, and may thus cause malfunction of the ubiquitin-proteasome protein degradation pathway.

To test this hypothesis, we have utilized HEK 293 cells stably transfected with a GFPu plasmid: Green Fluorescent Protein fused to an amino acid sequence known to target a protein to the proteasome. Proteasomal malfunction results in accumulation of GFP, and can be monitored by fluorescence detection. We have previously shown that proteasomal dysfunction caused by transient-co-transfection of polyQ-expanded AR and TG occurs androgen-dependently. It has been hypothesized that androgen-induced nuclear localization of the AR is responsible for the observed androgen-dependence of SBMA animal models. Transfection studies are underway to test this hypothesis, employing ARs in which the nuclear localization sequence has been deleted by removing amino acids 628-640 (NLS). These constructs do not enter the nucleus upon androgen introduction. Preliminary results may indicate transfection of polyQ-expanded NLS mutants, but not normal tract-length receptors cause proteasomal dysfunction. In addition, formation of protein aggregates is observed in cells which do not exhibit proteasomal dysfunction. Therefore nuclear localization is not critical, and androgens may induce a conformational change rendering the polyQ-expanded AR a better substrate for TG isopeptide bond formation, resulting in proteasomal dysfunction. In addition, formation of cytoplasmic protein aggregates does not contribute to proteasomal dysfunction.

**A dominant form of congenital stationary night blindness (adCSNB) in a large Chinese family: Exclusion of five known point mutations in three genes as the cause of the disorder.** *S.-H. Chen<sup>1</sup>, S. Zhuang<sup>2</sup>, S. Hu<sup>2</sup>, X. Liu<sup>3</sup>, B. Lin<sup>3</sup>, W. Wu<sup>3</sup>.* 1) Department of Pediatrics, University of Washington and Molecular Diagnostic Lab, Children's Hosp/Reg Med Ctr, Seattle, WA; 2) Hangzhou Hua-Da Genomic Center, Hangzhou, Zhejiang, P.R. China; 3) The School of Optometry and Ophthalmology, Wenzhou Medical College, Wenzhou, Zhejiang, P.R. China.

Congenital stationary night blindness (CSNB) is a group of eye disorders characterized by absence, or severe deficiency of night vision under dim illumination and is non-progressive in nature. Affected patients have been identified with autosomal dominant, autosomal recessive and X-linked forms of the disease. We have studied a large Chinese family with CSNB. The disorder has been continuously transmitted through at least 12 generations with over 40 known affected individuals. Based on the pattern of inheritance, the mode of inheritance is an autosomal dominant form (ad) of CSNB. We have obtained electroretinograms (ERG) from three patients and two normal individuals in this family. The ERG data revealed that the patients had diminished rod isolated responses in b-wave (10-20% of normal amplitude) to dim scotopic light (<1 cd/m<sup>2</sup>/s) under dark adaptation, and a decrease (~50% of normal amplitude) of a-wave and b-wave responses to bright light (24 cd/m<sup>2</sup>/s) stimulation following dark adaptation. Cone responses to single bright flash under light adaptation were normal. Cone responses to 30-Hz flicker had normal amplitude in the patients. The ERG data are consistent with the diagnosis of adCSNB. Five known point mutations in three different genes (rhodopsin, beta-cGMP phosphodiesterase and alpha-transducin) have been identified to be responsible for adCSNB in other (Caucasian) populations. We have amplified and sequenced exons containing the five known mutation sites of 16 affected and 14 unaffected individuals in this family. No mutation was found in any affected individuals. We conclude that the mutation causing the defect in this family must reside in another site or gene and thus is different from the ones found in Caucasian population.

**A Novel Compound Heterozygous Mutation in the Cellular Retinaldehyde-Binding Protein Gene (*RLBPI*) in a Patient With Retinitis Punctata Albescens.** *F.Y. Demirci*<sup>1</sup>, *B.W. Rigatti*<sup>1</sup>, *T.S. Mah*<sup>1, 2</sup>, *M.B. Gorin*<sup>1, 2</sup>. 1) Dept. of Ophthalmology, SOM, Univ. of Pittsburgh, Pgh, PA; 2) Dept. of Human Genetics, GSPH, Univ. of Pittsburgh, Pgh, PA.

Mutations in the gene (*RLBPI*) encoding the cellular retinaldehyde-binding protein (CRALBP) cause retinal dystrophy, variously termed as autosomal recessive retinitis pigmentosa, retinitis punctata albescens (RPA), Bothnia dystrophy, and Newfoundland rod-cone dystrophy. Recessive mutations in *RLBPI* (chr 15q26) seem to be an uncommon cause of retinal degeneration and only six CRALBP disease-causing mutations have been reported to date. The human CRALBP contains 316 amino acid residues (the initial Met is not present in the final protein) and functional studies suggest that ligand interactions are localized to C-terminal residues (120-313). A male patient presented with a clinical phenotype suggestive of RPA, characterized by numerous fine yellow-white dots in the retina. Using direct PCR sequencing, we screened the coding exons of *RLBPI* (exons 2-8) for mutations in genomic DNA sample obtained from this patient. We identified a novel compound heterozygous missense mutation of Gly145Asp (exon 5) and Ile200Thr (exon 6) in the *RLBPI* gene. Subsequent analysis of DNA samples obtained from his parents demonstrated that the two sequence alterations observed in proband's DNA were located on different alleles (each parent transmitted one alteration) and therefore both copies of the gene were altered (consistent with recessive disease). Analysis of 100 control chromosomes from normal subjects revealed no individuals with these sequence alterations. The *RLBPI* mutations identified in this study are likely to be pathogenic, based upon the prediction that these changes would have major effects on the protein by affecting the C-terminal domain, the variants were not found among normal controls, and they alter the evolutionary conserved residues among human, bovine, and mouse CRALBP orthologues. Identification of these novel mutations add to our understanding of the functional properties of CRALBP and the molecular basis of the retinal pathology associated with *RLBPI* defects.

**Evidence of autosomal dominant Leber congenital amaurosis (LCA) underlain by a CRX heterozygote null allele.**

*I. Perrault<sup>1</sup>, S. Hanein<sup>1</sup>, S. Gerber<sup>1</sup>, F. Barbet<sup>1</sup>, J.-L. Dufier<sup>2</sup>, A. Munnich<sup>1</sup>, J.-M. Rozet<sup>1</sup>, J. Kaplan<sup>1</sup>.* 1) Unité de recherches sur les Handicaps Génétiques de l'Enfant, INSERM U393, Hôpital des Enfants malades, Paris, France; 2) Service d'Ophtalmologie, Hôpital des Enfants Malades, Paris, France.

Leber congenital amaurosis (LCA) is characterized by a congenital blindness or greatly impaired vision in the first months of life. Hitherto, LCA was considered as an autosomal recessive genetically heterogeneous condition. Yet, the identification of de novo mutations in one LCA gene, CRX, opened the debate of a possible dominant inheritance in some LCA cases. Here, we report the transmission through two generations of an unambiguous LCA phenotype, accounted for by a heterozygote 1 bp deletion of the CRX gene, suggesting dominant inheritance of LCA in this pedigree. Considering the parental consanguinity of the index case in this family, the hypotheses of digenism or modifying effect of an other gene were very unlikely, supporting the view that in rare cases, LCA could be inherited as an autosomal dominant trait.

Program Nr: 2464 from 2003 ASHG Annual Meeting

**A *CRYGD* mutation associated with autosomal dominant cataract linked to chromosome 2q.** A. Shiels, D. Mackay. Ophthalmology/Visual Sciences, Washington Univ Sch Medicine, St Louis, MO.

Non-syndromic Mendelian cataract is a clinically and genetically heterogeneous lens disorder that most frequently presents as a sight-threatening autosomal dominant trait in childhood. Here we have mapped a locus for a dominant form of coral-like cataract to the gamma-crystallin gene (*CRYG*) cluster on chromosome 2q32-q36. Linkage analysis gave significantly positive two-point LOD scores ( $Z$ ) at markers D2S371 ( $Z = 3.81$ , recombination fraction [ $\theta$ ] = 0) and D2S369 ( $Z = 3.64$ ,  $\theta = 0$ ). Haplotype analysis indicated that the disease gene lay in the ~15 cM genetic interval between D2S1384 and D2S2248. Sequencing analysis of the *CRYG* cluster in an affected member of the pedigree failed to detect any significant nucleotide changes in the coding exons of *CRYGA*, *CRYGB* or *CRYGC*, however, we identified a heterozygous CA transversion in exon 2 of *CRYGD* that was predicted to result in the non-conservative, missense substitution of threonine for proline. Allele-specific PCR analysis confirmed that the mutant A allele co-segregated with affected but not unaffected members of the pedigree and excluded the CA transversion as a single nucleotide polymorphism in a panel of 170 normal unrelated individuals. This study suggests that a mutation in *CRYGD* underlies a historically important type of coralliform cataract first reported in 1895.

**NDP gene mutations in French Norrie families.** *G. ROYER*<sup>1</sup>, *S. HANEIN*<sup>2</sup>, *V. RACLIN*<sup>1</sup>, *N. GIGAREL*<sup>1</sup>, *J.-M. ROZET*<sup>2</sup>, *A. MUNNICH*<sup>1, 2</sup>, *J. STEFFANN*<sup>1</sup>, *J.-L. DUFFIER*<sup>3</sup>, *J. KAPLAN*<sup>1, 2</sup>, *J.-P. BONNEFONT*<sup>1, 2</sup>. 1) Genetics Department; 2) INSERM Unit U393; 3) Ophthalmology Unit, Hopital Necker-Enfants Malades, Paris, France.

Norrie disease is a rare and severe X-linked condition characterized by congenital blindness, and occasionally deafness and mental retardation in males. This disease has been ascribed to mutations in the Norrie disease protein gene (NDP) on chromosome Xp11.1. The function of the gene product remains unknown. Previous investigations of the two coding exons of NDP have identified largely sixty mutations so far. We analysed by direct sequencing the NDP gene in a series of twenty-one French families, eight of them being multiplex families. Thirteen mutations were detected in fourteen of the twenty-one families, including five intragenic deletions and eight point mutations. Six of them - namely, R38C, H43Q, V45M, IVS2-1G>C, R90C, and C128R - are hitherto unreported. Only one mutation was found to have arisen de novo. No NDP mutation was found in the seven remaining families. This observation raises the issues of misdiagnosis, phenocopies, or existence of other X-linked or autosomal genes, the mutations of which would mimic the Norrie disease phenotype.

**An exploration of Leber congenital amaurosis locus, allelic, and inheritance complexity.** *D.W. Stockton<sup>1</sup>, R. Quijano<sup>1</sup>, D. Nguyen<sup>1</sup>, M. Leppert<sup>2</sup>, R.A. Lewis<sup>1</sup>, J.R. Lupski<sup>1</sup>.* 1) Dept Molec & Human Genet, Baylor College Medicine, Houston, TX; 2) Eccles Institute of Human Genetics, University of Utah, Salt Lake City, UT.

Leber congenital amaurosis (LCA) is a clinically and genetically heterogeneous retinal dystrophy. Its causes have been partially unraveled at three mapped loci and at the molecular level through the discovery of six genes that, when mutated, result in LCA. Our data from sequencing every exon of all six testable genes in a cohort of LCA patients has revealed novel mutations that, like other published series, can explain the disease in a minority of cases. In many published reports, only a single heterozygous mutation is identified in genes associated with recessively inherited disease and attributed to cause the disease. Our comprehensive examination of each of these six genes for every individual raises doubt on this interpretation since, for nearly one third of families in which homozygous or compound heterozygous mutations were identified in one of the six genes, a heterozygous alteration was identified in one of the other genes. These third mutations do not appear to be necessary to cause disease and are being evaluated further for both segregation and modification of the phenotype. These mutations could alter significantly the interpretation of DNA analysis for diagnostic purposes.

**Optineurin and Juvenile Open Angle Glaucoma.** C.E. Willoughby<sup>1,2</sup>, L. Chan<sup>1</sup>, S. Herd<sup>1</sup>, G. Billingsley<sup>1</sup>, N. Noordeh<sup>1</sup>, A. Levin<sup>1</sup>, Y. Buys<sup>3</sup>, G. Trope<sup>3</sup>, E. Héon<sup>1</sup>. 1) Dept Ophthalmology and Vision Sciences, Hospital for Sick Children, Toronto, ON, Canada; 2) Dept of Medicine, University of Liverpool, Liverpool, UK; 3) Department of Ophthalmology, Toronto Western Hospital, Toronto, ON, Canada.

Sequence changes in *OPTN* (chr10p14-p15) at the GLC1E locus, were reported to cause or increase susceptibility to primary open angle glaucoma (POAG) and normal tension glaucoma (NTG). We assessed the role of *OPTN* in patients with juvenile open angle glaucoma (JOAG), in which the elevated intra-ocular pressures are associated with severe visual morbidity. Inclusion criteria: age of onset < 40 years, IOP > 22mmHg with optic nerve damage and visual field loss. Mutational analysis of *OPTN* used a combination of SSCP and direct sequencing. Sixty-six unrelated mixed ethnicity probands with JOAG were screened. Patients with *OPTN* sequence changes were further screened for *MYOC* sequence changes. A total of 10 sequence alterations were identified in subjects with glaucoma (JOAG and POAG) and control individuals. All sequence changes, except for H486R and A134A, were previously reported in POAG or controls. The c1767A>G nucleotide change (H486R) in exon 14 of *OPTN*, occurs in a highly conserved residue, and was seen in one patient with JOAG (1/66) and was not present in 101 control individuals nor in 107 POAG patients. A R545Q sequence change in exon 16 of *OPTN* was detected in one Chinese individual with familial JOAG and was not detected in 170 ethnically mixed control individuals but was present in 3/16 Chinese controls, suggesting R545Q may be an ethnic specific polymorphism. M98K was seen in 7/132 (5.3%) chromosomes of individuals with JOAG, in 8/96 (8.3%) chromosomes of patients with POAG and in 5/202 (2.4%) control chromosomes. The prevalence of the M98K polymorphism may confer a susceptibility risk to POAG ( $p>0.05$ ) but does not appear to predispose to JOAG. This is the first report of a potentially disease causing sequence alteration in *OPTN* in juvenile open angle glaucoma. Interestingly, the segregation of *OPTN* and *MYOC* mutations in one family, with both JOAG and POAG, is suggestive of an interaction between *MYOC* and *OPTN*.

**The (CGG)<sub>n</sub> repeat element within the 5'untranslated region of the *FMRI* message provides both positive and negative *cis* effects on *in vivo* translation of a downstream reporter.** L.-S. Chen, F. Tassone, P. Sahota, P.J. Hagerman. Biological Chemistry, University of California Davis, Davis, CA.

The human fragile X mental retardation 1 (*FMRI*) gene contains a polymorphic (CGG) trinucleotide repeat element in its 5'untranslated region (UTR). Expansion of the (CGG)<sub>n</sub> element beyond 200 repeats (full mutation range) generally leads to transcriptional silencing; consequent loss of the *FMRI* protein (FMRP) results in fragile X syndrome, the most frequent form of inherited mental retardation. For carriers of smaller expansions (54n200; premutation range), FMRP levels are gradually reduced with increasing repeat number, despite elevated *FMRI* mRNA levels, suggesting that translation is impeded within the premutation range. To examine in more detail the influence of the CGG repeat on translation, CMV immediate-early promoter constructs, containing the *FMRI* 5'UTR with various (CGG)<sub>n</sub> repeat lengths (0 n 99) and a downstream (luciferase) reporter, were transfected into two human lines, a neural cell-derived line (SK) and a fetal kidney cell-derived line (293). For both cell types, the CGG element exerts distinct effects on reporter expression, depending on the length of the repeat. For n 30, luciferase expression decreases with increasing repeat length, consistent with earlier observations of decreased FMRP expression in peripheral blood leukocytes over the same repeat range, despite a slight increase in mRNA level for the larger repeats. Surprisingly, for smaller alleles in the normal range (0 n 30), reporter expression actually increases by nearly two-fold with increasing repeat length in the absence of any change in mRNA level. These results suggest that the CGG repeat element can exert both positive (n 30) and negative (n 30) effects on translation. Interestingly, optimal translation appears to occur near the modal repeat number within the general human population.

This research is supported by NIH/NICHD grant HD40661.

**Detection of genomic rearrangements in the MECP2 gene by MLPA analysis.** *J. Herbergs<sup>1,3</sup>, C. Van Roozendaal<sup>1</sup>, E. Smeets<sup>1</sup>, D. Tserpelis<sup>1</sup>, C. De Die-Smulders<sup>1</sup>, A. Midro<sup>2</sup>, U. Moog<sup>1,3</sup>, C. Schrander-Stumpel<sup>1</sup>, H. Smeets<sup>1,3</sup>.* 1) Clinical Genetics, Academic Hosp Maastricht, Maastricht, Netherlands; 2) Clinical Genetics, Medical University of Bialystok, Poland;; 3) GROW, Maastricht University, The Netherlands.

Rett syndrome is an X-linked severe neurodevelopmental disorder predominantly affecting females. Genetic analysis of classical patients has shown that in approximately 80% of these cases the syndrome is caused by different mutations in the gene encoding methyl-CpG-binding protein-2 (MECP2). The routine DNA-diagnostic screening protocol mostly involves DHPLC, DGGE, SSCP or direct sequence analysis of the coding exons and immediate flanking intronic regions of the gene. These approaches however do not allow detection of gross rearrangements in the MECP2 gene. For this purpose we have analyzed 12 mutation-negative cases using a multiplex ligation-dependent probe amplification (MLPA, MRC-Holland) technique to allow the detection of deletions and/or duplications in the MECP2 gene. The tested cases involved 4 classical and 2 atypical Rett syndrome patients and a further 6 patients displaying some Rett-like suggestive symptoms. By means of this approach we identified gross rearrangements in 2 of the 4 analyzed classical Rett cases. In one case we observed a deletion of exon 3 and most of the coding region of exon 4. In the second case a duplication of exon 3 and the coding region of exon 4 was detected. Both rearrangement were subsequently verified and confirmed by Southern blot analysis. No aberrations were observed in any of the other cases. Our findings warrant further (MLPA) studies to establish the prevalence of large rearrangements in the MECP2 gene in mutation negative classical Rett syndrome cases. The MPLA technique could subsequently easily be implemented in the routine molecular screening protocol.

**Mutation analysis of MECP2 gene: Detection of three novel mutations and two novel polymorphisms. D.**

Zahorakova<sup>1</sup>, R. Rosipal<sup>1</sup>, J. Hadac<sup>2</sup>, N. Misovicova<sup>3</sup>, S. Nevsimalova<sup>4</sup>, J. Zeman<sup>1</sup>, P. Martasek<sup>1</sup>. 1) Department of Pediatrics, 1st Faculty of Medicine, Charles University, Prague, Czech Republic; 2) Department of Child Neurology, Thomayer University Hospital, Prague, Czech Republic; 3) Department of Clinical Genetics, University Hospital, Martin, Slovak Republic; 4) Department of Neurology, 1st Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic.

**Background:** Rett syndrome (RTT) is an X-linked dominant severe neurodevelopmental disorder caused by mutations in the methyl-CpG-binding protein 2 gene (MECP2). MeCP2 protein was identified by its ability to bind specifically to CpG-methylated DNA and is thought to act as a global transcriptional repressor. It contains two main functional domains, an N-terminal methyl-CpG-binding domain (MBD) followed by a transcriptional repression domain (TRD). There are 5 prevalent mutations known in the MECP2 that cause RTT. Four of them are detectable by restriction analysis. We report mutation analysis of 46 patients with clinical diagnosis of RTT from Czech and Slovak republics.

**Methods:** The whole coding sequence of MECP2 was amplified by PCR. Products were examined by restriction analysis and automatic direct sequencing. **Results:** The analysis revealed 15 different disease-causing mutations in 33 sporadic patients (72%). Three of them have not been previously published: a missense mutation - P302S, a small deletion of 3 bp - 1069delAGC, and the deletion of 172 bp along the insertion of 41 bp - 1063del172bp+ins41bp. Fifteen patients had missense mutations (R133C, K135E, T158M, R306C), fourteen patients carried nonsense mutations (Y141X, R168X, S204X, R255X, R270X, R294X), and one had a frameshift mutation (1157del41bp). Two novel polymorphisms, 587 C>G (T196S), and 815 C>T (P272L) were detected in patients carrying mutations R133C, respectively R255X. **Conclusions:** Our results facilitate the diagnosis of RTT at the molecular level in the Slavonic population, and provide insight into the molecular pathology of Rett syndrome. *Supported by GAUK 301/01/P068..*

**Reduced expression of proteolipid protein 2 in XLMR patients with mild mental retardation.** *T. Wang<sup>1</sup>, L. Zhang<sup>1</sup>, C. Obie<sup>1</sup>, P. Boytner<sup>2</sup>, C. Schwartz<sup>2</sup>, A. Wright<sup>3</sup>, J. Trent<sup>4</sup>, D. Valle<sup>1</sup>.* 1) Inst. of Genet. Med., Johns Hopkins Univ., Baltimore, MD; 2) Greenwood Genetic Center and Clemson Univ., SC; 3) MRC Hum. Genet. Unit, Western General Hosp., Edinburgh, UK; 4) NHGRI, Bethesda, MD.

X-linked Mental retardation (XLMR) occurs in 1 in 600 males and is genetically heterogeneous. Approximately 40 genes have been cloned among the estimated 150-200 responsible loci on the X chromosome. Using a human X chromosome-specific cDNA microarray, we screened lymphoblast cell lines from 32 XLMR males and identified a candidate gene that encodes proteolipid protein 2 (PLP2). Significant reduction (~6-8 fold) of PLP2 mRNA was confirmed by Northern blot in lymphoblasts in three patients and PLP2 protein was not detectable by Western blot. DNA sequencing revealed a C>A mutation in the proximal promoter of PLP2 that alters the core binding site for Ets-domain transcription factors. The same mutation was detected in another young XLMR male with mild mental retardation with an overall IQ of 69. Moreover we found this mutation co-segregates with mild mental retardation in a four-generation X-linked pedigree with MR and RP. Linkage analysis in this family mapped the responsible gene to Xp11.23, the location of PLP2. We are screening a large collection of control males to determine if this mutation is associated with MR phenotype. PLP2 is a 17 kD membrane protein with 4 trans-membrane domains and shares similar physical properties but not sequence homology with PLP1, the gene responsible for Pelizaeus-Merzbacher disease. In a related work we found that a mis-sense mutation in PLP2 may be associated with retinal degeneration. RT-PCR study showed that PLP2 is expressed in multiple tissues including brain and retina. In situ hybridization revealed PLP2 expression in cerebral cortex and especially in the hippocampus. By immunohistochemistry and con-focal microscopy, we localized PLP2 protein to the ER membrane in cultured cells. Using chimeric mutant PLP2 promoter-luciferase reporter gene constructs in HEK293 cells, we found the mutant promoter reduced the transcription by 3-4 folds. These results suggest that reduction of PLP2 expression may be associated with XLMR.

**Chromosomal abnormalities in male infertility: Screening and diagnosis. A PRELIMINARY STUDY IN THE SOUTH OF TUNISIA.** *A. Bahloul<sup>1</sup>, N.B. Abdelmoula<sup>2</sup>, A. Amouri<sup>3</sup>, A. Saad<sup>4</sup>, L. Keskes<sup>2</sup>, T. Rebai<sup>2</sup>.* 1) Service d'urologie, CHU H. Bourguiba, Sfax, Tunisia; 2) Laboratoire d'Histologie Embryologie, Facult de Mdecine de Sfax, Tunisia; 3) Laboratoire de Cytogntique, Institut pasteur Tunis, Tunisia; 4) Laboratoire de Cytogntique, Hpital F. Hached Sousse.

Chromosome investigations have shown that about 2-10% of infertile men has an abnormal karyotype. Sex chromosome abnormalities predominate and the most prevalent anomaly is 47,XXY (Klinefelter's syndrome) with a frequency of 60%. We report a cytogenetic study that was carried out in 182 patients. The aim of this preliminary prospective study was to detect chromosomal abnormalities on infertile men with sperm count less than 10 million. X chromatin investigations were carried out for screening of numerical X chromosome abnormalities, classical cytogenetic analysis was conducted mainly by R banding and FISH analysis was carried out when it was necessary. The results of this study showed an overall incidence of 10,5% chromosome abnormalities. Cytogenetic abnormalities predominantly concern the X chromosome (56%), including Klinefelter's syndrome and 46,XX males, but also rearrangements such as deletions and reciprocal translocations. Results of our preliminary study confirm the elevated impact of numerical abnormalities of X chromosome in males with azoospermia . It suggest that both sex chromatin and chromosomal analysis should be considered as a routine examination in men with severe oligospermia or azoospermia. This enables access to genetic counseling and testing, which can be facilitated by their assisted reproduction providers. Having genetic risk information allows these patients to make educated, thoughtful decisions about their reproductive options.

**Usefulness of cytogenetics in female infertility.** *F. Ben Mrad<sup>1</sup>, N.B. Abdelmoula<sup>1</sup>, A. Amouri<sup>2</sup>, M. Mnif<sup>3</sup>, W.E. Abbes<sup>3</sup>, A. Saad<sup>4</sup>, M. Abid<sup>3</sup>, T. Rebai<sup>1</sup>.* 1) laboratoire d'histologie, faculte de medecine de sfax, Sfax, Tunisia; 2) laboratoire de cytogenetique, institut pasteur de tunis, Tunisia; 3) laboratoire de cytogenetique, CHU F. Hached Sousse, Tunisia; 4) Service d'endocrinologie, CHU H. Chaker Sfax, Tunisia.

Female factor infertility are less clearly defined but women with premature ovarian failure are at increased risk for being carriers of structural or numerical sex chromosome abnormality. Women who have mosaicism for sex chromosome aneuploidy are also observed to repeated failed IVF cycles. The association of recurrent spontaneous abortion with parental chromosome abnormalities has long been established. There is approximately a 5-6% risk that either the male or the female is a carrier of a chromosome abnormality. These are frequently structural abnormalities such as robertsonian translocations, reciprocal translocations, or inversions. POF-related abnormalities range from the complete absence of one X chromosome to assorted deletions and translocations to mutations in specific genes. Potential molecular mechanisms include both dominant and recessive mutations in X-linked genes as well as nonspecific chromosome effects that impair meiosis. A list of candidate X-linked POF genes is emerging from molecular studies of X chromosome abnormalities. We report a prospective study that was carried out in all patients affected by premature ovarian failure with secondary amenorrhea and referred, since 1999, to the endocrinology service of the Hedi Chaker university hospital of Sfax. First to establish the prevalence of chromosome abnormalities we performed cytogenetic analyses. In this study cytogenetic abnormalities predominantly concern the X chromosome, including mosaic Turner syndrome, but also rearrangements such as deletions. Mutational analysis of candidate genes in these women with idiopathic POF is needed to determine which gene contribute to the cause of this disorder.

**Prolonged prenatal exposure to magnesium sulfate associated with bone abnormalities mimicking genetic bone disease.** *J.M. Kogan<sup>1</sup>, K.E. Wedig<sup>2</sup>, J.A. Whitsett<sup>2</sup>, E.K. Schorry<sup>1</sup>*. 1) Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Division of Neonatology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Two female former 30-week premature triplets (triplets B and C) were evaluated at 3 weeks of age for multiple fractures and bone abnormalities to rule out genetic bone disorder. The infants were conceived by IVF to a 32 y.o. G2P0Ab1 mother. Pregnancy was complicated by premature rupture of membranes of triplet A at 20 weeks. Preterm labor beginning at 22 weeks was treated with brethine, sulindac, and indocin, as well as magnesium sulfate at 2.5g/hr for 8.5 weeks. The babies were delivered by C-section for suspected chorioamnionitis and polyhydramnios. The infants were intubated and treated with surfactant. Triplet A died on the first day of life with sepsis. At 3 weeks of age, x-rays of triplets B and C revealed thin, demineralized bones with multiple fractures of the ribs, humeri, and clavicle. Skull bones were demineralized, with no wormian bones noted. Triplet C also had the appearance of hemi-vertebrae on spine X-rays. Examination was significant for very wide fontanelles, and white sclera. Initial lab values included normal calcium and phosphorus, mildly elevated magnesium, and elevated alkaline phosphatase for both infants. Phosphoethanolamine levels were normal. The bone abnormalities were presumed to be related to prolonged prenatal exposure to magnesium, with genetic bone disease unlikely. The infants developed BPD and were discharged to home on oxygen as well as a high calcium/phosphorus formula, however follow-up DEXA scans at 3 months age showed persistent low bone density.

Previous reports have shown a causal relationship between prolonged magnesium tocolysis and abnormal fetal bone mineralization. The exact mechanism is unknown but has been hypothesized to be related to fetal parathyroid gland suppression. Our patients showed a more severe degree of demineralization and greater number of fractures than other reported cases. This case points out the necessity of including prolonged magnesium therapy in the differential diagnosis of the newborn infant with multiple fractures.

**Prenatal Diagnosis of Osteogenesis Imperfecta Type 3.** *C. Maxwell<sup>1,2</sup>, A. Toi<sup>3</sup>, S. Keating<sup>4</sup>, M. Thomas<sup>1</sup>, D. Chitayat<sup>1,2,5</sup>.* 1) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Univ. of Toronto, Toronto, Canada; 2) Dept. of Obstetrics and Gynecology, Division of Maternal Fetal Medicine, Mount Sinai Hospital, Univ. of Toronto, Toronto, Canada; 3) Dept. of Medical Imaging, Mount Sinai Hospital, Univ. of Toronto, Toronto, Canada; 4) Dept. of Laboratory Medicine and Pathobiology, Mount Sinai Hospital, Univ. of Toronto, Toronto, Canada; 5) Department of Pediatrics, Division of Clinical Genetics and Metabolism, The Hospital for Sick Children, Univ. of Toronto, Toronto, Canada.

Osteogenesis imperfecta (OI) type 3 is a non-lethal, progressively deforming skeletal dysplasia often recognized after birth due to short stature and antenatal fractures. Inheritance is usually autosomal dominant.

On fetal ultrasound, findings include demineralization and fractures resulting in shortening and angulation of long bones with absence of narrow chest and rib fractures. We report three cases of OI type 3 presenting with bowed or angulated long bones on fetal ultrasound.

Case 1: A 19 week ultrasound revealed bent femora, tibiae, fibulae and humeri measuring below the 5th percentile. The skull was indented with external pressure. Post termination X-rays showed slight demineralization, asymmetrical healing fractures of the long bones and thin ribs.

Case 2: A 19 week ultrasound revealed all long bones measuring below the 3rd percentile and bent femora. Post termination X-rays showed asymmetrical fractures of long bones more pronounced in the lower limbs.

Case 3: A woman with known OI type 3 presented at 17 weeks of gestation. Fetal ultrasound revealed short femora and humeri and absent calvarial ossification. The long bones showed marked angulation at sites of healing fractures. The chest was not deformed. DNA analysis confirmed the diagnosis.

Differentiation of OI type 3 from OI type 2 is important for genetic counseling as the former is non-lethal. Shortening and asymmetrical deformity of long bones less severe than in OI type 2 with relative sparing of chest and ribs suggest the diagnosis.

**Twins and Heart Defects: a retrospective study of 27 cases.** *P. Eydoux<sup>1</sup>, V. Cusin<sup>2</sup>, J.F. Oury<sup>3</sup>, A.L. Delezoide<sup>4</sup>, A. Azancot<sup>5</sup>.* 1) Cytogenetics, Children's and Women's Hospital, Vancouver, BC, Canada; 2) Biochimie Genetique, Hospital Robert Debre, Paris, France; 3) Obstetrics and Gynecology, Hospital Robert Debre, Paris, France; 4) Biologie du Developpement, Hospital Robert Debre, Paris, France; 5) Cardiologie Foetale, Hospital Robert Debre, Paris, France.

Congenital heart defects (HD) are among the most prevalent malformations in liveborns. Twin studies have demonstrated a strong genetic component of HD, which are more frequent in monozygotic (MZ) than dizygotic (DZ) twins. In the former, heart malformations may be discordant despite identical genotype and similar prenatal environment, resulting in a relatively low concordance rate. In reported series, results are often impaired because of insufficient data to identify the HD and to determine zygosity. In this retrospective study, we report prenatal and postnatal data on 27 sets of twins diagnosed during fetal life by echocardiography, in which zygosity was established using morphological and molecular criteria. Between 1990 and 1999, 33 HD were detected in 27 twin pregnancies. All fetuses had a karyotype and all fetuses with a cono-truncal HD were tested for microdeletion 22q11. DNA was analyzed from amniotic fluid cells or from placenta de-paraffinated specimens. Whenever intra-uterine fetal death (IUFD) or termination of pregnancy occurred, complete macroscopic examination of the fetus and placenta was performed. Out of 33 HD, 9 were type I, 16 were type II, 2 were type III, 3 were type IV and 3 type VI. Sex ratio of affected fetuses seemed close to 0.5 except for type III and VI, for which only female were affected (n=5). Our sample was skewed towards MZ pregnancies, mostly monochorionic (MC) diamniotic (DA). Sixteen IUFD occurred. Overall, MZ pregnancies were more frequent in our sample. The most frequent HD was type II (half of the cases). HDs were frequently discordant in MZ pregnancies and always discordant in DZ pregnancies. An excess of females was seen in DC pregnancies, and most IUFDs occurred in MZ pregnancies and in males. Monosomy X was a frequent finding in MZ pregnancies. The significance of these findings will be discussed.

**Polymorphisms in matrix metalloproteinase-2 are associated with increased risk of developing thoracic aortic aneurysm.** *D. Guo*<sup>1</sup>, *Z. Yin*<sup>1</sup>, *Y. Qi*<sup>1</sup>, *A. Estrera*<sup>2</sup>, *H. Safi*<sup>2</sup>, *S. Shete*<sup>3</sup>, *T. King*<sup>1</sup>, *D. Milewicz*<sup>1</sup>. 1) Dept Internal Medicine, Univ Texas/Houston Med Sch, Houston, TX; 2) Dept of Cardiovascular Surgery, Univ Texas/Houston Med Sch, Houston, TX; 3) Dept of Epidemiology, Univ of Texas M. D. Anderson Cancer Center, Houston, Texas.

Metalloproteinases (MMPs) are a family of proteinases that play a role in remodeling vascular extracellular matrix in both normal and pathological states, including aortic aneurysm. MMP2 was expressed at elevated levels in thoracic aortic aneurysm and dissection (TAAD). To understand the genetic contribution to the development of sporadic TAAD, we genotyped single nucleotide polymorphisms (SNPs) located in MMP2 and performed case-control association studies. Eight SNPs in MMP2 were selected from the Celera database along with a function SNP that had been reported. These SNPs were genotyped in 166 Caucasian controls and 297 Caucasian TAAD patients. Two of these SNPs showed to be significantly associated with TAAD. Allele G of MMP2-2 and allele C of MMP2-3 showed significant association with TAAD ( $p=0.01$ ). For MMP2-2, the odds ratio (OR) was 1.44 (95% confidence interval (C.I.) 1.08-1.92) for allele G as compared to allele A and similarly for MMP2-3, the OR was 1.44 (C.I. 1.09-1.90) for allele C as compared to allele T. Similarly, for MMP2-2, the OR was 1.88 (C.I. 1.05-3.38) for individuals who carried genotype GG compared to those carried genotype AA and for MMP2-3, the OR was 2.01 (C.I. 1.18-3.45) for individuals carried genotype CC compared to those carried genotype TT. Interestingly, in male population, individuals carried genotype CC in MMP2-3 had a significantly higher risk of developing TAADs compared to those carried genotype TT. The haplotypes of MMP2-2 and MMP2-3 were constructed. The haplotype frequencies were significantly different between TAAD and control with a  $p$ -value of 0.03. The individuals who had the homozygous GC genotype showed significantly higher risk of developing TAAD compared to individuals who had the homozygous AT genotype. The  $p$ -value is 0.02 and OR is 2.322 (C.I. 1.14 4.73). Our results suggest that MMP2 could be implicated as the susceptibility genes for development of TAADs.

**In utero methotrexate exposure resulting in long bone aplasia.** *D.J. Zand<sup>1</sup>, C. Blanco<sup>2</sup>, B. Coleman<sup>2</sup>, D. Huff<sup>3</sup>, E. Zackai<sup>1</sup>.* 1) Dept Genetics, CHOP, Philadelphia, PA; 2) Dept Radiology, Hospital of the University of Pennsylvania, Philadelphia, PA; 3) Dept Pathology, CHOP, Philadelphia, PA.

Methotrexate has been increasingly utilized to control autoimmune disease, as an anti-neoplastic agent, and as an abortifacient. Fetal exposure has been reported to result in facial and digital dysmorphology similar to that observed in fetal aminopterin syndrome, a structurally related folic acid antagonist. Megalencephaly, cranial ossification defects, hypertelorism, prenatal growth deficiency, and post-axial polydactyly are some of the most consistent fetal findings. We report two cases where methotrexate was used unsuccessfully as an abortifacient. Ultrasound and fetal MRI imaging suggested digital and facial dysmorphia consistent with methotrexate exposure. Both pregnancies were electively terminated based on prenatal radiographic findings. Ultrasound evaluation of the fetus in Case 1 showed the radius and ulna to be shortened and bowed, while the femora were notably absent. Hypertelorism and microcephaly were also present. Cardiac echo noted double outlet right ventricle (DORV) pathology. In Case 2, skeletal anomalies included bilateral absence of radii and fibulae. Frontal bones were asymmetric and a large parietal foramina was present. Post mortem evaluation noted an absent thymic isthmus, accessory spleens, and facial dysmorphia including hypertelorism, bulbous nasal tip and high arched, prominent eyebrows. There were only three post axial digits and a thumb on both hands. To our knowledge, this is the first report of long bone aplasia associated with in utero methotrexate exposure. In our two cases, either fibulae or radii were involved bilaterally. This affect may correlate with the high methotrexate dose required for abortifacient use. These two cases emphasize that prenatal ultrasonographic monitoring is vital for evaluation of pregnancies exposed to methotrexate.

**THE IMPACT OF PRENATAL DIAGNOSIS ON THE BIRTH PREVALENCE OF MALFORMED CHILDREN IN A REGION WITHOUT UPPER LIMIT FOR TERMINATION OF PREGNANCY.** *B. Dott, Y. Alembik, M.P. Roth, C. Stoll.* Medical Genetics, Hopital de Hautepierre, Strasbourg, France.

Objectives : to describe the impact of prenatal diagnosis on the birth prevalence of congenital anomalies (CA) during 22 years in a well defined population. Material : 293,923 consecutive pregnancies of known outcome. The study was divided into 3 periods 79-88, 89-94 and 95-2000. In the area under study there is no upper limit for termination of pregnancy (TOP). Results : During the 3 periods of the study the livebirth (LB) prevalence of the malformed per 10,000 decreased for some CA : central nervous system (16.3, 11.6, and 12.2), neural tube defects (5.6, 2.3, and 2.9) musculoskeletal system (51.6, 48.5, and 45.6), transposition of great arteries (5.0, 3.4, and 3.7), and Down syndrome (DS) 21 (9.8, 10.7, and 6.2) but increased for other : encephalocele (0.68, 0.86, and 1.04), urinary system (36.5, 60.7, and 63.2), congenital heart defects (67.3, 79.0, and 71.2), respectively whereas the prevalence rate of TOP increased for all categories of CA. For non chromosomal CA the prevalence rate of TOP for 1000 total births/year increased from 0.9 to 1.8 and to 2.5, respectively. For DS the ratio LB/TOP was 5.1, 1.3, and 0.4, respectively. The total number of malformed fetuses/children increased in parallel to the increase of the number of TOPs. The gestational age at TOP decreased dramatically for DS. However for non chromosomal CA it was the opposite as TOP was performed after 24 weeks of gestation in 42%, 66%, and 74% of the cases, respectively. This evolution of the prevalence rate of LB from 1979 to 2000 was the result of more efficient but late prenatal diagnosis on one hand and of the increase of maternal age, (the 35-37 years-old group involved 6% of the TOP in 1979-1988 and 17% of the cases in 1995-2000). In conclusion in a region where there is no upper limit for TOP, these last two decades, despite more efficient prenatal diagnosis and an increasing number of TOPs the LB prevalence of malformed children decreased for only some categories of CA. Except for chromosomal anomalies, the number of late TOPs, after 24 weeks of gestation, was increasing.

**Use of microarray technology to identify differential gene expression in Down syndrome placentae: validation of methodology.** *P. Dar, J.C. Ferreira, K.C. Livne, J. Segal, D. Khabele, B.L. Gross, S.J. Gross.* Dept OB/GYN, Albert Einstein Col Medicine, Bronx, NY.

Background: Detection rates for prenatal maternal screening for Down syndrome (DS) has improved over the past decade, although false positive rates remain a clinically significant issue. Genome wide gene scanning has the potential to ultimately identify more specific markers for DS. Using microarray technology, we previously identified 7 genes that were overexpressed in pooled second trimester DS placentae compared to controls. Purpose: The aim of this present study was to validate the findings from our microarray experiments using Quantitative Real Time RT-PCR (QPCR) in an independent set of placental samples. Methods: Samples of DS (n=8) and control (n=6) second trimester placentae were obtained anonymously under an IRB approved protocol. RNA was extracted and reverse transcribed to cDNA aliquots, which were amplified with QPCR as per our standard protocol. The gene expression ratios of DS vs. controls were calculated using the Student t-test. Results:

Gene/EST	N31645	KRT8	PLAC3	AA001841	ALDH7	KISS-1	PTTG1IP
DS/N Ratio	4.6	5	16.6	7.2	1.2	6.7	1.1
P value	<0.05	<0.05	<0.05	<0.05	NS	<0.05	NS

Conclusions: This study confirms our initial premise that microarray technology can be used for genome wide detection of differentially expressed genes in aneuploid placentae and that independent validation is necessary. Further study of confirmed genes and their protein products can be used to develop a more precise panel of markers and further our understanding of the underlying pathogenic mechanisms in DS.

Program Nr: 2481 from 2003 ASHG Annual Meeting

**Gene expression profiles of the TNF-related apoptotic pathway in Down Syndrome placentae.** *S.J. Gross, P. Dar, K.P. Livne, J.C. Ferreira, B.L. Gross, D. Khabele.* Div Reproductive Genetics, Dept OB/GYN, Albert Einstein Col Medicine, Bronx, NY.

**Background:**Down syndrome (DS) is the most common aneuploidy identified at birth. Apoptotic mechanisms have been implicated in the phenotypic features of this syndrome. TNF superfamily ligands and their receptors have been identified in human placentae and are thought to play a major role in both the normal and pathologic development of this organ. Characteristic clinical and pathological sequelae of DS may be related to abnormal placental development. **Purpose:**The purpose of this study was to determine if genes related to the TNF apoptotic cascade are differentially expressed in DS placentae.**Methods:**Samples of DS (n=6) and control (n=6) second trimester placentae that were matched for gestational age were obtained anonymously under an IRB approved protocol. RNA was extracted and reverse transcribed to cDNA aliquots which were amplified with QPCR, using sequence specific primers for TNF-related apoptosis-inducing ligand (TRAIL), TNF-, TRAIL death receptor DR4 and TRAIL decoy receptors DcR1 and DcR2. The association between gene expression in DS (cases vs. controls) for each gene was calculated using the Student t-test (significance at  $p < 0.05$ ).**Results:**TRAIL expression was significantly upregulated in DS placentae compared to controls (DS expression level 163.3, control expression level 52.1,  $p$  value=0.0004). The remaining four genes did not show significant differential expression. **Conclusions:**TRAIL was significantly overexpressed in DS placentae compared to controls while TNF-alpha as well as the three TRAIL receptors evaluated were not. Further studies are required to characterize the expression and determine the biological function of TRAIL and other genes whose function may modulate TRAIL activity.

**Trisomy 8 pseudomosaicism in amniocytes: difficulties in prenatal diagnosis and counseling.** *S. Carter*<sup>1</sup>, *P. Dar*<sup>1</sup>, *V. Pulijaal*<sup>1</sup>, *B. Gross*<sup>2</sup>, *C. Salafia*<sup>2</sup>, *S. Gross*<sup>1</sup>. 1) Reproductive Genetics, Montefiore Medical Ctr, Bronx, NY; 2) Bronx Lebanon Hospital, Bronx, NY.

Trisomy 8 mosaicism is a rare disorder with extreme phenotypic variability. It is characterized by mild to moderate mental retardation, dysmorphic facial features, skeletal malformations, and cardiovascular anomalies. Additionally, the literature has reports of individuals with mosaicism for trisomy 8 cells who have normal intelligence. This wide phenotypic presentation presents a challenge for genetic counseling when this chromosome complement is detected prenatally. Another confounding factor is that prenatal diagnosis of this pseudomosaicism has resulted in anomalous newborns with true mosaicism.

Here we report a case of prenatal diagnosis of trisomy 8 pseudomosaicism following a positive MSAFP3 screen for Down syndrome in a 20-year-old G3P0020 biracial female. The family and pregnancy history were unremarkable. Amniocentesis was performed without complications and the limited fetal ultrasound was normal. The amniocentesis results revealed 46, XX (35 cells)/47,XX,+8 (1 cell), type B pseudomosaicism. A detailed ultrasound performed at 21 weeks gestation demonstrated normal fetal anatomy and appropriate interval growth. After genetic counseling, the patient decided to terminate the pregnancy because of the uncertain neurodevelopmental prognosis. Fetal tissue was obtained for cytogenetic analysis and a detailed postmortem examination was performed.

We present the post termination results, summarize the literature for case management guidelines, and discuss the complexity of counseling for this rare mosaicism.

**Application of the multi-PRINS technique for simultaneous detection of chromosomes 18, X and Y in uncultured amniocytes.** *M. Gadji, K. Krabchi, M. Bronsard, R. Drouin.* Human and Molecular genetic research center, Medical biology, Laval University, Quebec, PQ, Canada.

**Objective:** Comparative evaluation of the multi-PRINS (primed in situ labeling) technique for simultaneous detection of chromosomes 18, X and Y in uncultured amniocytes for prenatal diagnosis of aneuploidy. **Methods:** The multi-PRINS technique for simultaneous detection in the same cells of three chromosome targets, 18, X and Y, was performed according to the procedure described by Yan J et al. *Chromosoma* 109:565, 2001. Oligonucleotides were annealed to their specific individual chromosome targets, followed by in situ elongation using Taq DNA polymerase to incorporate labeled dUTPs. The sites of the newly synthesized DNA sequences were revealed as fluorescent signals using immunochemical reaction. The fluorescent signals corresponding to chromosomes 18, X and Y were displayed as yellow, red and green color spots, respectively. Multi-FISH using commercial chromosome 18, X and Y probes was carried out for comparison. Two persons, one blinded, examined all slides. Fifty cases were analyzed using both multi-PRINS and multi-FISH. Between 50 and 200 nuclei were scored for each case for each technique. To compare the sensitivity and specificity of PRINS and FISH, Fisher's exact test was carried out using the observed distribution of cells showing one or more signals. **Results:** In all cases, there was no significant difference in the detection of chromosomes 18, X and Y regarding the sensitivity, the specificity and the efficiency. Both multi-PRINS and multi-FISH yield similar distribution of the number of spots per nucleus. Both techniques were able to identify aneuploid cases without ambiguity. The entire PRINS procedure, including the harvest time, could be completed in less than 5 hours vs. FISH in 24 hours. **Conclusion:** Multi-PRINS can accurately and reliably detect aneuploidies involving chromosomes 18, X and Y in uncultured amniocytes as well as can multi-FISH. In addition, multi-PRINS represents a faster and more cost-effective alternative to FISH for prenatal testing of aneuploidy in uncultured amniocytes.

**Multiple fetal anomalies including holoprosencephaly caused by 7q monosomy/5q trisomy in the offspring of a double translocation carrier.** *L. Mehta*<sup>1</sup>, *A. Yenamandra*<sup>2</sup>, *P. Koduru*<sup>2</sup>, *J. McLaughlin*<sup>1</sup>, *R. Schiff*<sup>1</sup>, *N. Vohra*<sup>3</sup>, *B. Rochelson*<sup>3</sup>. 1) Medical Genetics & Cardiology, Schneider Children's Hospital at North Shore; 2) Cell Genetics; 3) Maternal-Fetal Medicine, North Shore University Hospital, Manhasset, N.Y.

A 35 y.o. woman was found to have fetal sonogram findings consistent with alobar holoprosencephaly (HPE); a single orbit, absent nasal structures and a supraorbital proboscis were present. Echocardiogram revealed a large inlet-VSD and a small mid-muscular VSD. The couple have had a healthy child, two miscarriages and an infant with anencephaly who died at birth. Fetal karyotype on amniocentesis was initially reported as normal 46,XX. It was then determined that the father carried two apparently balanced chromosome translocations, t(1;8)(q32;q21.1) and t(5;7)(q35;q36). Given the subtle terminal breakpoints of the (5;7) translocation, FISH and high resolution studies were done on amniocytes. It was confirmed that the fetus was monosomic for 7q36-ter and trisomic for 5q35-ter. The anomalies present are consistent with this imbalance. Deletion of terminal 7q causes haploinsufficiency of the SHH gene located at 7q36 (HPE3 locus). Terminal 7q deletions are consistently reported with variable degrees of HPE and other malformations but anencephaly is not reported; there were no documented chromosome studies on the anencephalic infant. Trisomy for terminal 5q is reported with dysmorphisms, growth and developmental delays and heart defects. The presence of two translocations in one individual, as in the father, is rare. Notably chromosome 7 is involved in three reported double translocation cases; in one such case, two fetuses with HPE inherited 7q32-ter monosomy from the carrier father. Our pt. opted to continue the pregnancy (as she did the previous anencephalic pregnancy). It was of note that the father's chromosome result was not divulged, and was only ascertained through records. Without this information, the subtle fetal chromosome imbalance would have been missed. This case highlights the importance of subtelomeric FISH testing in unexplained multiple malformation syndromes, particularly with a suspicious family history.

**Down syndrome with pure partial trisomy 21q22 due to a paternal insertion (4;21) uncovered by amniotic fluid interphase FISH.** *D. Hopcus-Niccum<sup>1</sup>, J.R. Stanley<sup>2</sup>, S.A. Vaz<sup>2</sup>, J.J. Mulvihill<sup>1</sup>, S. Li<sup>1</sup>.* 1) Department of Pediatrics, OUHSC, Oklahoma City, OK; 2) Prenatal Assessment Center, Department of Obstetrics-Gynecology, OUHSC, Oklahoma City, OK.

FISH analysis to screen aneuploidies of the selected chromosomes X, Y, 13, 18 and 21 on fetal cells utilizing commercial probes (AneuVysion, Vysis, USA) is becoming a standard test despite rare false-positive or false-negative results. A 32-year-old Caucasian woman had amniocentesis at 18 weeks and 4 days gestation because of a positive screen for Down syndrome and a slightly increased nuchal fold (0.58 cm). Overnight FISH was performed and all thirty nuclei analyzed had two signals of X chromosome and three signals of chromosome 21. The diagnosis of a female fetus with Down syndrome was made. Seven days later, routine chromosome analysis showed all 15 cells had a modal number of 46 chromosomes, including two X chromosomes. Neither an extra chromosome 21 nor a Robertsonian translocation was found. A relatively larger, but subtle, light band (4q21), was noticed on one homologue; later, it was confirmed that part of 21q22 was inserted into the band of 4q21. Chromosome analysis of both parents revealed that the father carried an apparently balanced insertion (4;21). To further determine the size of the inserted chromosome 21 segment, FISH analysis utilizing a locus specific probe (AML1) and a chromosome 21 paint probe was performed. An AML1 gene signal was clearly visualized on the derivative chromosome 4. Hybridization of the chromosome 21 paint probe showed that approximately one third of the whole long arm of chromosome 21 was inserted in the band 4q21. The karyotype of the fetus was defined as 46,XX,der(4)ins(4;21)(q21;q22.13-q22.2). Genetic counseling was provided regarding the implication of the laboratory findings and the couple decided to continue the pregnancy. The baby was delivered without complication at 40 weeks gestation. As expected, the baby has typical features of Down syndrome. In this case, without knowing of the presence of three signals of 21q, this fetus could have been a missed diagnosis or a difficult determination of the origin of this white extra chromosome material.

**PGD for aneuploidy on 513 embryos from 56 initiated cycles using laser assisted embryo biopsy and FISH significantly increases implantation rates, reduces miscarriages, and decreases multiple births.** *W.G. Kearns<sup>1</sup>, J. Graham<sup>2</sup>, T. Han<sup>2</sup>, J. Carter<sup>2</sup>, A. Davis<sup>2</sup>, K.S. Richter<sup>2</sup>, E. Widra<sup>2</sup>, M.J. Levy<sup>2</sup>.* 1) Center for Preimplantation Genetics, Rockville, MD; 2) Shady Grove Fertility, Rockville, MD.

PGD was performed to increase implantation rates (IR), reduce miscarriages and reduce multiple births following in vitro fertilization (IVF). Patients underwent PGD due to 2 recurrent miscarriages or unsuccessful IVF cycles. The control group comprised couples with 2 unsuccessful IVF cycles undergoing routine IVF. PGD was performed on blastomeres from 513 cleaving embryos from 56 initiated cycles prior to 44 embryo transfers. Embryo biopsy and FISH were used to determine aneuploidy for chromosomes 13, 14, 15, 16, 17, 18, 21, 22, X and Y. One blastomere from each 4-9 cell embryo was analyzed. Twenty-four percent (125/513) of embryos were diploid and 72 embryos were transferred for 44 couples. Fifteen percent (76/513) of embryos were not diagnosed because of cell fragmentation. Aneuploidy was found in 61% (312/513) of embryos tested. No transfer occurred for 12 couples due to a chromosome abnormality in all embryos analyzed.

Age	PGD IR	Controls IR
< 38 yrs	38% (SE=9)	22% (SE=2)
38 - 40yrs	36% (SE=17)	13% (SE=2)
> 40yrs	7% (SE=4)	5% (SE=2)

Thirty-nine percent (17/44) of women achieved a clinical pregnancy with an IR of 28% (20/72). PGD significantly increased ( $p < 0.05$ ) the IR for women under 41. PGD also overall reduced the miscarriage rate from 15% (23/153) to 6% (1/17) and the percent of multiple births from 28% (40/142) to 12% (2/17) compared to controls.

**High incidence of unbalanced conceptuses from segregation of a paternal balanced translocation 7q34;9q34.1.**

*S.B. Olson<sup>1,4,5</sup>, H. Lawce<sup>4</sup>, N. Ouhibi<sup>2</sup>, C. Davis<sup>4</sup>, C. Durum<sup>4</sup>, J. Lira<sup>4</sup>, S. Kelson<sup>4</sup>, M.L. Sorensen<sup>5</sup>, P. Stenzel<sup>3,5</sup>, M. Vahdati<sup>4</sup>, Y. Akkari<sup>1,4,5</sup>.* 1) Dept Mol & Med Genet, Or Hlth & Sci Univ(OHSU), Portland, OR; 2) Dept OB/GYN, OHSU, Portland, OR; 3) Dept Path, OHSU, Portland, OR; 4) Cytogenet Lab, OHSU, Portland, OR; 5) School of Med, OHSU, Portland, OR.

Useful figures regarding risks to specific balanced translocation carriers of producing unbalanced gametes and offspring are scarce. Early data from preimplantation genetic diagnosis (PGD) studies suggest the incidence of unbalanced conceptuses in these families to be higher than previously estimated. We report the cytogenetic analysis of 4 fetuses and 11 embryos in a family with a paternal balanced rearrangement. The couple was ascertained following an abnormal maternal serum screen with abnormal ultrasound at 16 weeks gestation (increased nuchal fold of 5.2 mm; bilateral pyelectasis). Amniocyte chromosome analysis, followed by parental karyotyping, revealed a 46,XX,der(9)t(7;9)(q34;q34.1)pat karyotype. A follow-up ultrasound at 20 weeks gestation showed increased nuchal translucency (3.8 mm), bilateral club feet and a complex heart defect. The pregnancy was terminated. Autopsy further delineated the heart anomaly as double outlet right ventricle with side-by-side great vessels and subaortic VSD, hypoplastic left heart with patent mitral valve, bicuspid pulmonic valve and bicuspid aortic valve. Three subsequent pregnancies were unbalanced, including another with der(9) and two with der(7). Three of the 4 fetuses presented with increased nuchal translucencies. Autopsy was refused for the second der(9). One der(7) fetus had bilateral club feet with no other anomalies; the other had as sole findings small kidneys and adrenals with normal histology. PGD was also performed on 11 embryos; 10 of these were unbalanced reflecting at least 6 different segregation products of the translocation; one balanced embryo implanted and resulted in spontaneous loss. This study illustrates the high incidence of unbalanced conceptuses in balanced translocation families and emphasizes the importance of thorough genetic counseling in making informed reproductive decisions.

**Prenatal detection of *de novo* balanced chromosomal rearrangements: a follow-up study.** M. Shago<sup>1,6</sup>, W.S. Meschino<sup>3</sup>, S.A. Farrell<sup>4</sup>, D. Chitayat<sup>2,6</sup>, E.J.T. Winsor<sup>5,6</sup>. 1) Departments of Paediatric Laboratory Medicine and; 2) Clinical Genetics, Hospital for Sick Children; 3) Department of Genetics, North York General Hospital; 4) Department of Genetics, Credit Valley Hospital; 5) Pathology and Laboratory Medicine, Mount Sinai Hospital; 6) University of Toronto, Toronto, Canada.

Prenatal detection of *de novo* balanced chromosomal rearrangements presents a dilemma for expectant parents. Whereas familial balanced rearrangements are generally without clinical manifestations, *de novo* rearrangements are associated with an increased risk of mental retardation (MR) and/or congenital anomalies. Based on a study by Warburton (1991), risk values of 6.7% and 9.4% for *de novo* translocations and inversions respectively are frequently provided to parents. The present study is a chart review of 61 patients with prenatal diagnosis of a *de novo* apparently balanced reciprocal translocation or inversion. Our goal was to document decisions made by parents, and to provide information on long term follow-up of children identified prenatally with *de novo* balanced rearrangements. Patients were identified by searches of cytogenetic databases from 1988 to present. Outcome of pregnancies was livebirth 44%, termination 32%, intrauterine demise 4%, and unknown 20%. Postnatal outcome information was available for 39 of the pregnancies. Age at last follow-up ranged from birth to 11 years. In the translocation group two children with abnormalities were identified: one had severe expressive language disorder and one had developmental delay with intractable seizures. In the inversion group one child with multiple congenital anomalies and one child with a hydronephrotic kidney were identified. Excluding cases ascertained due to ultrasound abnormalities, the risk for phenotypic/developmental abnormality was 8% in the *de novo* translocation group (n=25) and 20% in the *de novo* inversion group (n=10). Although this study provides further evidence that there is an increased risk for abnormality associated with *de novo* apparently balanced chromosomal rearrangements, a larger case-controlled study is necessary to obtain a more accurate risk.

Program Nr: 2489 from 2003 ASHG Annual Meeting

**Tandem direct duplication of 8p22-23.1 associated with Dandy-Walker variant.** *P.Z. Reingold, P. Dar, S.M. Carter, S. Klugman, P.L. Wilmot, V.R. Puligaal, S.J. Gross.* Dept of OB/GYN. Albert Einstein College of Medicine/Montefiore Medical Center. Bronx, NY.

Background: Inverted duplication 8p is one of the most common duplications observed and ranks in frequency with the other classical deletion syndromes, such as Wolf-Hirschhorn and cri-du-chat. It is characterized by profound mental retardation commonly related to agenesis of corpus callosum and/or Dandy-Walker malformation, facial dysmorphism, and malformations of kidneys and heart. Tandem direct duplication of the 8p22-23.1 segment, however, is a rare finding. In the small number of cases thus far reported isolated mental retardation was the most prevalent finding in the absence of associated anomalies. Case report: We describe a 32 year old G2P1000 pregnant patient who was referred to our genetic counseling clinic for a positive maternal serum screening test for Down syndrome. Her medical history was unremarkable and her obstetrical history was significant for full term intrauterine fetal death thirteen years prior to current pregnancy. First trimester screening revealed a nuchal translucency in the normal range but abnormal maternal serum biochemical markers levels (betaHCG 2.16MOM and PAPP-A 0.38MOM). Second trimester maternal serum quad screen was positive for Down syndrome (Risk of 1:65). Chromosome analysis and FISH revealed a 46XX, dir dup(8) (p22p23.1) karyotype. Detailed sonographic anatomy scan performed at 22 weeks revealed an isolated absence of the inferior vermis, consistent with the diagnosis of Dandy-Walker variant. Following a thorough genetic counseling the patient opted to continue with the pregnancy. Comment: This rare case implicates a possible association between tandem direct duplication of the 8p22-23.1 and Dandy-Walker variant. Detailed sonographic evaluation of the fetal posterior fossa should be offered to patients carrying a fetus with such karyotype.

**Chromosome abnormalities among fetuses with pleural effusions detected on prenatal ultrasound examination: Comparison of aneuploidy rates between isolated pleural effusions and those with other congenital anomalies.** *K. Waller, T. Custer, A. Donnenfeld.* Genzyme Genetics, Dallas, TX, Santa Fe, NM, and Philadelphia, PA.

**Purpose:** To determine the frequency of chromosome abnormalities in fetuses with prenatally detected pleural effusions and to compare the aneuploidy rate between pregnancies with isolated pleural effusions and those with additional anomalies. **Methods:** A retrospective analysis of our database for amniotic fluid samples submitted with an indication of pleural effusion was performed. **Results:** Amniotic fluid analysis was performed on 250 samples in which pleural effusion was identified as an indication for chromosome analysis. In 95 of these samples (38%), pleural effusion was the only fetal abnormality identified as an indication for chromosome analysis. The remaining 155 fetuses had other associated anomalies identified in addition to pleural effusion (complex pleural effusions). No cell growth occurred in 2 cases among the isolated pleural effusions and in 7 cases among the complex pleural effusions. Eight significant chromosome abnormalities were identified in the isolated pleural effusions (8.6%) including five cases of trisomy 21, one case of trisomy 18, one mosaic trisomy 20, and one mosaic unbalanced translocation. Among the complex pleural effusion cases, there were 74 chromosome abnormalities (30.4%). 37 had 45,X, 26 had trisomy 21, 5 had trisomy 18, two had mosaic Turner syndrome, two had unbalanced translocations, one had trisomy 13 and one had triploidy. The difference in aneuploidy rate between the isolated pleural effusions and the complex effusions was statistically significant  $P < 0.0001$  by Wald Chi-Square test. The estimated odds ratio was 0.091 indicating that chromosome abnormalities are 11.03 times more likely among fetuses with complex pleural effusions than isolated pleural effusions. **Conclusions:** Chromosome analysis is warranted following the detection of a pleural effusion on prenatal ultrasound examination. The aneuploidy rate in fetuses with isolated pleural effusions (8.6%) is significantly less than among fetuses with complex pleural effusions (30.4%) ( $p < 0.0001$ ).

**SLAMDUNC-a very successful new community program for first trimester combined screening for Down syndrome.** *L.J Sheffield<sup>1,2,3</sup>, R. Williamson<sup>1,2,3</sup>, J.L. Halliday<sup>2,4</sup>, R.M. Forbes<sup>1</sup>, A.M. Jaques<sup>2,3</sup>, A. Cronin<sup>1,2</sup>, L. Bonacquisto<sup>1</sup>, I. Francis<sup>1,2</sup>.* 1) Genetic Health Services Victoria,; 2) Murdoch Childrens Research Institute; 3) Department of Paediatrics, University of Melbourne; 4) Victorian Birth Defects Register, Department of Human Services, Melbourne, Australia.

We describe the first community based application of first trimester combined screening for Down syndrome in 18140 pregnancies. Our program uses a Single Laboratory And Many Diagnostic Ultrasound Nuchal-translucency Centres (abbreviated SLAMDUNC). The ultrasound is done in many specialised private obstetric ultrasound practices in the community. The results are better than when the ultrasound and biochemistry are done in a single tertiary centre as in an OSCAR (One Stop Counselling Assesment and Risk) clinic. SLAMDUNC detected 60/64 (93.8%) of the cases of Down syndrome for a false positive rate of 5%. If the detection rate is fixed at 85% then the false positive rate is 2.1% which compares very favourably to the 6% false positive of the SURUSS study for the combined test and the 1.3% false positive test of the integrated test. We also show for the first time, that the accuracy of the test is significantly greater if the blood sample is collected before 13 weeks. These state of the art results were achieved by using well-trained obstetric ultrasonologists, a very experienced screening laboratory and a team of genetic counselors, clinical geneticists and clerical staff. This presentation shows how the program worked to achieve the achievements of an OSCAR clinic but taking the ultrasound test out of tertiary centre into the community.

**Invasive trophoblast antigen (ITA) as a marker of fetal aneuploidy in the first and second trimester of pregnancy.** *U. Sancken*<sup>1</sup>, *R. Schemer*<sup>2</sup>, *M.R. Pandian*<sup>3</sup>, *J.E. Lee*<sup>3</sup>. 1) Institute of Human Genetics, University of Goettingen, Goettingen, Germany; 2) Nichols Institute Diagnostics, Bad Vilbel, Germany; 3) Quest Diagnostics Nichols Institute, San Juan Capistrano, CA.

ITA is a hyperglycosylated isoform of human chorionic gonadotropin (hCG) that has been reported to be increased in trisomy 21 (Down syndrome) affected pregnancies. In order to confirm these findings, we are conducting a prospective study of pregnant women undergoing routine prenatal screening at 10 to 18 weeks of gestation. To date, ITA concentrations have been determined in 2,500 urine and 5,000 serum samples. Pregnancy outcomes are being obtained from cytogenetic analysis of amniotic fluid or phenotypic observations following birth. Here we report early results. Between the 10th and 14th weeks of gestation, ITA levels decreased sharply, after which they decreased gradually to a plateau at 16 weeks of gestation. Serum ITA levels were dependent on maternal weight at time of specimen collection ( $ITA = 5.43 - 0.0152 * \text{weight}$ ; weight in kg). Although pregnancy outcomes are still being gathered, so far we have identified 18 aneuploid cases. The table below shows the distribution of serum ITA results in these cases.

Centile	Trisomy 21	Trisomy 18	Triploid	Turner	Klinefelter
>95th	6/7	0/3	0/2	0/3	0/3
<5th	0/7	3/3	2/2	0/3	0/3

Conclusion: Our preliminary results indicate that ITA may be a useful marker for prenatal detection of fetal aneuploidy (eg, trisomies 21 and 18). Accurate assessment of gestational age, especially in early gestation, and correction for maternal weight will be required when interpreting ITA concentrations.

**Y chromosome heterochromatin of differing lengths in two cell populations of the same individual.** *H. Lawce<sup>1</sup>, Y. Akkari<sup>1,2</sup>, S. Kelson<sup>1</sup>, C. Smith<sup>1</sup>, C. Davis<sup>1</sup>, M. Vahdati<sup>1</sup>, S.B. Olson<sup>1,2</sup>.* 1) Clin. Cytogenetics Lab, Oregon Health & Science Univ, Portland, OR; 2) Dept. Mol. and Med. Genetics, Oregon Health & Science Univ, Portland, OR.

The human Y chromosome is known to contain a heterochromatic region on the distal q arm comprising two highly repetitive sequence families, DYZ1 and DYZ2. This region is variable in length in different male populations without known clinical consequence. In this report, we present an unusual case of the prenatal diagnosis of a fetus with two populations of the Y chromosome: one with a short block of heterochromatin (Yqh-) and one with a longer block of heterochromatin (Yqh+). These two populations were further studied using fluorescent in situ hybridization (FISH) and quinacrine banding (Q-banding). Both Y chromosomes appeared to be structurally normal by these analyses. The biological father, to date, has been unavailable for study. Subsequent ultrasound examination at 20 weeks gestation revealed male genitalia. Based on these findings and the present understanding of this region of the Y chromosome, the family was counseled to expect no clinical impact of the two different Y chromosome-bearing cell populations. The baby was delivered at term without complications; the phenotype appeared normal. Follow-up post-natal blood chromosome analysis confirmed the presence of the two cell populations. The child is progressing normally at 9 months of age. This mosaicism may be explained by a post-zygotic simple deletion or unequal crossover event between sister chromatids in the DYZ region.

**Prognostic Significance And Phenotype Correlation In Infertile Men With Yq Microdeletions.** *R. Dada<sup>1</sup>, N.P. Gupta<sup>2</sup>, K. Kucheria<sup>1</sup>*. 1) Anatomy, AIIMS, New Delhi, India; 2) Urology, AIIMS, New Delhi, India.

Microdeletion of the long arm of the Y chromosome, are associated with spermatogenic failure and have been used to define three regions on Yq (AZFa, AZFb and AZFc) which are critical for spermatogenesis. These 3 loci act at different stages of germ cell development and deletion of each loci result in a characteristic phenotype. Deletion of AZFa, AZFb, AZFc results in Sertoli Cell Only syndrome (SCO), maturation arrest and hypospermatogenesis respectively. One hundred and seventy five infertile males with idiopathic oligozoospermia and azoospermia were included in this study. Cytogenetic and semen analysis was done in each case. Testicular Fine Needle aspiration Cytology was collected whenever possible. Of the 175 cases, 40 cases were klinefelter or variants. In cytogenetically normal cases microdeletion analysis was done using STS-PCR approach using primers sY84, sY86 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc). Eight of the 102 cases showed deletion of at least one of the AZF loci. Four cases had AZFc deletion, three cases had AZFa and AZFb deletion and one case showed AZFb deletion alone. Two cases with AZFa and AZFb had SCO Type 1 syndrome and 2 cases of AZFc deletion showed hypospermatogenesis and 1 case showed maturation arrest. The FSH and LH levels were elevated in these cases. Variation in testicular phenotype in cases with AZFc deletion is due to multiple copies of the gene and presence of autosomal genes. Thus various factors genetic, epigenetic and environmental modulate the effect of these genes. Cases with AZFc microdeletion show a progressive decline in semen quality thus these cases are counselled to go in for semen cryoconservation at the earliest age should they opt for ICSI at a later date. Deletion on Y chromosome make the Y chromosome more prone to secondary larger deletions resulting in worsening of testicular phenotype. Therefore detection of Yq microdeletions encompassing the AZF loci determines the prognosis and management of these infertile cases.

**Common Arterial Trunk in the fetus: characteristics, associations and outcome in a multicenter series of 23 cases.** *M. Gentile*<sup>1,2</sup>, *D. Paladini*<sup>3</sup>, *M. Marasini*<sup>4</sup>, *A.L. Buonadonna*<sup>1</sup>, *M.G. Russo*<sup>3</sup>, *A. Di Carlo*<sup>1</sup>, *F. Cariola*<sup>1</sup>, *M. Vassallo*<sup>3</sup>, *P. Volpe*<sup>2</sup>. 1) Dept Medical Genetics, IRCCS S de Bellis, Castellana (BA), Bari, Italy; 2) Neonatal-Maternal Health Department, ASL BA/04, Bari, Italy; 3) Dept. Obstetrics and Gynecology, University Federico II, Naples; 4) Dept. Pediatric Cardiology, IRCCS Gaslini, Genova.

Objective: To assess: A) the accuracy of prenatal diagnosis; B) the incidence of extra-cardiac and chromosomal anomalies; C) the perinatal outcome in a population of 23 fetuses diagnosed with common arterial trunk (CAT), seen at three referral centers. Design: Observational trial of 23 fetuses with a confirmed diagnosis of CAT. All of them underwent fetal echocardiography, detailed anatomic scan and karyotyping. In 19/23 cases, FISH analysis was performed to detect the 22q11 microdeletion. The following variables were retrieved from databases and evaluated: gestational age at diagnosis, anatomic variants of the CAT, the presence of extra-cardiac and chromosomal anomalies, pregnancy and fetoneonatal outcome. Results: prenatal diagnosis of CAT proved correct in 23/24 cases, the last case being a pulmonary atresia with ventricular septal defect (PAVSD). A second cardiovascular anomaly was present in 8 cases (34.8%). Extra-cardiac anomalies were found in 10 cases (43.4%). FISH analysis showed the presence of the 22q11 microdeletion in 6/19 cases (31.6%). Pregnancy and fetoneonatal outcome was as follows: 8 (34.8%) terminations of pregnancy, 2 (8.7%) intrauterine deaths, 5 (21.7%) post-natal deaths (before or after surgery). The remaining 8 (34.8%) neonates are alive and thriving after surgery (6 cases) or awaiting surgery (2 cases). Conclusions: CAT can be reliably diagnosed and characterized in prenatal life, although differentiation from PAVSD is sometimes challenging. The association with chromosomal anomalies is consistent (8.7%), but there is a higher risk of 22q11 microdeletion (31.6%), in agreement with post-natal studies. The relatively poor survival rate (34.8%) is due to the high rate of terminations and the unfavorable cardiac anatomy in some cases.

**PCR quantitation of fetal cells in maternal blood is significantly compromised by presence of cell free fetal DNA.**

*X. Zhong, W. Holzgreve, S. Hahn.* Dept. Ob/Gyn, Univ Basel, Basel, Switzerland.

Fetal cells enriched from the blood of pregnant women still presents an enticing alternative for the development of a safe and efficacious non-invasive method for prenatal diagnosis. However, although promising in small studies, most enrichment methods used to date have failed to realise the long sought after goal. These failures have raised the questions of whether the enrichment methods were sub-optimal or whether there were no fetal cells present in the maternal blood sample being analysed. In order to answer these issues we have attempted to address a problem which has long beset research in this field, namely that no reliable method exists to determine the frequency of fetal cells in a given maternal blood sample. As we have considerable experience with the use of the Taqman real-time quantitative PCR system, we have now used this technology to address this task. In our analysis we used both a single copy gene on the Y chromosome (SRY) as well as a multi-copy sequence (DYS14). Our examination of cell free fetal DNA in maternal plasma samples indicated a very good concordance regarding the determination of fetal sex and the concentrations of cell free fetal DNA. We next addressed the issue of the number of fetal cells in maternal blood. As the concentration of cell free fetal DNA in maternal blood is much higher than fetal cell numbers we were concerned that the presence of the high levels of cell free fetal DNA may compromise the PCR quantitation. Hence, we examined paired samples which had been washed extensively or from which the plasma had been removed by standard centrifugation. Our study showed that the number of fetal genome equivalents detected in the unwashed samples was indeed significantly higher than in the washed samples. Therefore previous analyses may have been misled by this feature. We, furthermore, observed that fetal cells are too scarce to be detected by the use of single copy gene sequences such as SRY, and that even with the use of high copy sequences such as DYS14, their numbers did not exceed 2 fetal cells per mL of maternal blood.

**Prenatal Diagnosis of Trisomy 3 Mosaicism.** *A. Zaslav<sup>1</sup>, G. Pierno<sup>2</sup>, A. Fougner<sup>2</sup>, J. Jacob<sup>1</sup>, R. Kazi<sup>1</sup>, D. Blumenthal<sup>3</sup>, S. Sturim<sup>2</sup>, M. Shaham<sup>4</sup>, J. Davis<sup>5</sup>, J. Fox<sup>3</sup>.* 1) Dept of Laboratory Medicine, Long Island Jewish Medical Ctr, The Albert Einstein College of Medicine, New Hyde Park, NY; 2) SonoScan Genetic Sciences, Forest Hills, NY; 3) Dept of Peds, Schneider Children's Hosp, The Albert Einstein College of Medicine, New Hyde Park, NY; 4) Genzyne Genetics, Yonkers, NY; 5) Weill College of Medicine/Cornell University, New York, NY.

Trisomy 3 mosaicism has rarely been reported. We present a prenatally diagnosed case of trisomy 3 mosaicism. Amniocentesis was performed for AMA. The karyotype was 47,XX,+3[8]/46,XX[27]. Trisomy 3 was found in eight cells from three different culture vessels, meeting the criteria for true mosaicism. A repeat amniocentesis confirmed mosaicism for trisomy 3 (i.e., 47,XX,+3[1]/46,XX[18]). PUBS was performed at 19 weeks and showed 46,XX in 100 cells. A high-resolution ultrasound was performed at 18 weeks and fetal echocardiogram was performed at 20 weeks and neither revealed abnormalities. The infant was delivered at 34 weeks and had a normal examination, with the exception of symmetric IUGR. Apgar scores were 8 and 9 at one and five minutes, respectively. Chromosome analysis of 50 cells each from peripheral blood, placenta and umbilical cord revealed 46,XX in all cells. There was no evidence of trisomy 3 mosaicism. At 6 months the baby was progressing normally. Only two prenatally diagnosed cases of mosaic trisomy 3 have been reported. One resulted in a normal live born male. The other case resulted in an abnormal live born male with multiple congenital anomalies who died at 18 months due to congenital heart disease. Trisomy 3 was confirmed in skin fibroblasts and amnion cultures, whereas blood cultures were normal in 100 cells counted (Hsu, et al., 1997, *Prenat Diag* 17(3):201-242). The absence of trisomy 3 cells postnatally in our patient indicates that the trisomy 3 mosaicism was most likely placental, which resulted in IUGR. Prenatally diagnosed chromosome mosaicism is problematic for both patient and clinician. This is only the third report of trisomy 3 mosaicism. Additional cases of prenatally diagnosed mosaicism for rare trisomies are necessary to more accurately assess the significance of these findings.

Program Nr: 2498 from 2003 ASHG Annual Meeting

**Giant Omphalocele: Prenatal Diagnosis, Short and Long Term Outcomes.** *R.D. Wilson, J.M. Biard, M.P. Johnson, H.L. Hedrick, A.W. Flake, T.M. Crombleholme, S.N. Adzick.* Ctr Fetal Diag/Treatment/Wood, Children's Hosp Philadelphia, Philadelphia, PA.

**Objective:** To review prenatal features, short and longterm outcomes in prenatally diagnosed giant omphaloceles (GO) managed at a single institution (1996-2001).

**Study Design:** This retrospective study evaluated prenatal features and short and long term outcomes in pregnancies referred with prenatal diagnosis of GO. Parents and medical providers were surveyed by written questionnaire about the child's health . Quality of life(PedsQL4.0)tool was used in the older children . IRB 2002-2-2683 was obtained.

**Results:** GO was evaluated in 17 pregnancies ( 8 live born, 4 termination , 2 IUFD, 3 no follow-up). Complete extra-abdominal liver within peritoneal sac, ascites and extreme levocardia were prenatal findings in the survivors . Live born cases were delivered by C section at 37 weeks gestation with a mean BW 2903 gms. All neonates required intubation. Two infants died within 1 year. Four of the 6 survivors had respiratory insufficiency (mean ventilation 76 days, range 12-101 days ). Respiratory and feeding problems (first oral feed mean 250 days , range 15-577 days) complicated the early neonatal period. Long term follow-up was available for 5 patients (mean 33.2 months ). Asthma, recurrent pulmonary infections, feeding problem, gastroesophageal reflux, and failure to thrive were the major concerns. Both male survivors had bilateral inguinal hernias requiring repair. Developmental delay was present in 2 of 5 patients. Mean hospital stay for survivors was 148.4 days (range 23-316).

**Conclusions:** Respiratory and feeding problems were the most common neonatal and longterm management issues. Parents need to be aware of the probability of multiple surgeries and long hospitalization following birth.

**Evaluating Genetic Counseling Methods and Attitudes of Obstetrician/Gynecologists in Austria.** *V. Moser, M.A. Spence, M. Bocian.* Dept Pediatrics, Univ California, Irvine, Orange, CA.

Austria has no specially trained genetic counselors. This study surveyed the attitudes and practices of obstetrician/gynecologists in Austria regarding prenatal genetic counseling. A questionnaire consisting of demographics and eighteen questions was distributed to 931 Austrian obstetrician/gynecologists. A total of 269 (28.9%) respondents' questionnaires were returned and analyzed. Prenatal counseling in Austria is similar to that in America with regard to content and ideology, although not as standardized or thorough as that conducted by masters level genetic counselors. A three or more generation family history is seldom done (only 16% always do so) and the majority of physicians (56%) do not ask about their patients ethnicity. Only 76% of respondents explain normal male/female karyotypes to a patient when discussing amniocentesis. Most respondents felt confident overall in their role as providers of genetic information, although the majority also thought that the field of genetics is expanding too rapidly to keep up (67%). The majority felt competent to explain Down syndrome (83%), spina bifida (80%) and cystic fibrosis (58%) to patients (etiology, prognosis, recurrence risk), although more respondents indicated difficulty with cystic fibrosis (20% versus ~5% for each Down syndrome and spina bifida). When asked whether Austria already has sufficient expert personnel to address all the needs and questions of patients, the majority (74%) of respondents indicated their belief that Austria has enough resources, which appears contrary to the finding that 67% felt genetics is expanding too rapidly to keep up. The results of this study suggest a need in Austria for better training, continuing education, and standardization of practice in prenatal genetic counseling to cope with the rapidly increasing changes in genetics. Austria would also benefit from genetics experts, such as masters trained genetic counselors, as primary and/or secondary contact persons for at-risk patients.

**PRENATAL SCREENING FOR DOWN SYNDROME IN THE HISPANIC POPULATION.** *J. Santolaya-Forgas, J. De Leon-Luis.* TTUHSC, Amarillo, TX.

Our objective was to determine if Hispanics had similar first (free-bhCG, PAPP-A) or second trimester (AFP, hCG and uE3) maternal serum values than Caucasians. We have evaluated 200 pregnant women who had consented to donate blood for biochemical research early in pregnancy. We excluded 56 patient because they developed obstetrical complications. 144 patient reached term and had appropriate for gestational neonates. 123/144 and 129/144 patients had donated their blood at first and second trimester, respectively. All results were expressed as MoMs for gestational age. 62% were Caucasian, 29% Hispanic, 4% African-A. and 5% of other cultural backgrounds. Table 1 shows summary statistics in mean (S.D.).

MATERNAL AGE	MATERNAL WEIGHT	AGE AT FIRST TRIMESTER	AGE AT SECOND TRIMESTER
24 (5) years	86 (18.8) Kg	11.9 (1.5) weeks	16.8 (1.3) weeks
AGE AT DELIVERY	NEONATAL WEIGHT	APGAR	pH UMBILICAL VEIN
39.1 (1.1) weeks	3338.5 (420) grs	9 (0.5)	7.3 (0.07)

Table 2 shows mean values expressed in MoM for Caucasians and Hispanics.

	PAPP-A	Free Beta h CG	AFP	HCG	UE3
CAUCASIAN	1.5	1.5	1.11	1.27	0.97
HISPANIC	1.1	0.8	1.13	1.23	1.16
p-VALUE	0.26	<0.01	0.82	0.75	<0.02

These results suggest that the risk for Down syndrome might be underestimated in the Hispanic population using current first and second trimester algorithms. Further studies are recommended.

**Response to a newly formed University Genetic Screening Program in a USA semi-rural area.** *V.P. Noble, R. Yebra, I. Galn, J. De Leon-Luis, J. Santolaya-Forgas.* OB/GY, TTUHSC, Amarillo, Te.

Maternal age (MA), maternal serum markers (MS) and ultrasound (U/S) are commonly used prenatal screening procedures. Our aim was to evaluate acceptance rate for US-guided diagnostic testing after a positive MA, MS or U/S screening in our population. A single geneticist attends a population of 180,000 people with ~ 4200 deliveries/year. We report on 736 pregnant women referred for prenatal genetic counseling between 2000-2002 (1324 total consultations). The cultural backgrounds of the study population was: 62% European; 31% Hispanic; 4 % African-American and 3% other. 47% of patients had private insurance (P) and 53% were on Medicaid (M). 299 (40.6%) patients were referred due to family history of genetic disorders, mental retardation or congenital anomalies, recurrent miscarriage, maternal conditions such as diabetes, exposure to teratogens, etc., and for the purpose of this study were excluded from further analysis.

	(N)	P	M	%TOTAL PREG. POPULATION	ACCEPTANCE PREN. TESTING		
					P	M	
MA	174	122	52	174/8500 (2%)	35 %	30 %	(p. less than 0.05)
MS	128	43	85	128/8500 (1.5%)	37 %	35.2 %	(ns)
U/S	135	62	73	135/8500 (1.6%)	29 %	31.5 %	(ns)

The lower than expected referral rate for any of the prenatal screening categories may reflect a combination of lack of genetic education and limited availability of genetic services in our community prior to October 2000. After non-directive genetic counseling the acceptance rate for further prenatal testing is similar to that of other inner city USA University centers.

**Pre-implantation genetic diagnosis for Sanjad-Sakati syndrome.** A. Al-Aqeel<sup>1, 2</sup>, C. Serdar<sup>3</sup>, N. Sakati<sup>2</sup>, K. Jarodi<sup>3</sup>, P. Ozand<sup>2</sup>, A. Hellani<sup>3</sup>. 1) Dept Pediatrics, Riyadh Military Hosp, Riyadh, Saudi Arabia; 2) Dep of Genetics, King Faisal Specialist Hospital & Research Centre, Riyadh Saudi Arabia; 3) Dep of Reproductive Medicine, King Faisal Specialist Hospital & Research Centre, Riyadh Saudi Arabia.

The Sanjad-Sakati syndrome (SSS), MIM 241410 is an autosomal recessive disorder characterized by congenital hypoparathyroidism, growth and mental retardation. In Saudi Arabia the disease is caused by a deletion of 12bp of Tubulin Cofactor E (TBCE gene), starting from nucleotide 156 until nucleotide 166. In a Saudi family with two affected siblings with SSS, pre-implantation genetic diagnosis (PGD) was undertaken, fluorescent PCR (F-PCR) was performed on DNA showing heterozygosity and homozygosity status of the parents and the affected children. F-PCR was then used on a single cell using one round of amplification. Our allelic drop out (ADO) in lymphocyte was 0% since all the cells showed matched diagnosis. Our blastomere diagnosis showed 5 heterozygous, 3 homozygous and 3 normal, a mendelian percentage reflecting a negligible ADO. One heterozygous and one normal embryo were transferred because of their very good quality (morula). A singleton pregnancy was ensured and prenatal diagnosis by CVS confirmed a normal fetus. This is the first PGD for SSS families. Moreover, this is the first report showing negligible ADO in one round of F-PCR without linked markers.

**Prenatal diagnosis of Brachmann-de Lange syndrome using 2-D and 3-D ultrasonography.** *L. Applewhite<sup>1</sup>, B. Rochelson<sup>1</sup>, J. McLaughlin<sup>2</sup>, N. Vohra<sup>1</sup>, M.G. Bialer<sup>2</sup>.* 1) Divisions of Maternal Fetal Medicine and; 2) Medical Genetics, North Shore Univ Hosp/NYU Med Center, Manhasset, NY.

A 26 year old woman was referred at 21 wk gestation for comprehensive ultrasound (U/S) after a screening U/S reported fetal edema, possible ascites and a small left ventricle of the heart. The pregnancy had been essentially uncomplicated. There had been a urinary tract infection treated with Keflex. A triple screen had been performed which gave a risk for open neural tube defects of 1 in 260 and a Down syndrome and trisomy 18 risk of 1 in 5000. The couple were not consanguineous and the family history was not significant. The 2-D U/S findings included frontal bossing, micrognathia, thickened nuchal fold, diffuse skin edema, diaphragmatic hernia, shortening of the fetal long bones with missing fingers, pleural effusion and a VSD. The differential diagnosis discussed with the couple included trisomy 18, Brachmann-de Lange syndrome (BDLS) and Miller syndrome. 3-D U/S confirmed the above findings and showed flexion contractures of the upper extremities with severe oligodactyly and facial dysmorphisms including ocular hypertelorism, anteverted nares, micrognathia and downturned mouth. Amniocentesis was performed and chromosomes were normal 46,XY. The patient chose to terminate the pregnancy at 21 wk 3 days by induction to permit an examination of the fetus. Findings included ocular hypertelorism, synophrys, low-set posteriorly rotated ears, anteverted nares, flat nasal bridge, downturned mouth and webbed neck. The forearms were hypoplastic with no visible hand and a single finger bilaterally. There were bilateral elbow pterygia. The features were diagnostic of BDLS. There have been several previous sonographic prenatal diagnoses of BDLS using 2-D U/S. Diaphragmatic hernia with limb anomalies are suggestive of this diagnosis. In this case, the 3-D U/S assisted in making the diagnosis by revealing dysmorphic features that would be difficult to determine on 2-D. 3-D U/S can help the perinatologist and geneticist make a prenatal diagnosis and improve the counseling of the patient.

***De novo* mutation in the 21-hydroxylase gene detected in two patients with congenital adrenal hyperplasia (CAH): implications for complexity in genetic diagnosis of the disease.** R. Mao<sup>1</sup>, J. McDonald<sup>1</sup>, M. Cantwell<sup>2</sup>, W. Tang<sup>1</sup>, K. Ward<sup>1</sup>. 1) DNA Diagnostic Laboratory, University of Utah School of Medicine, Salt Lake City, UT; 2) Obstetrical Diagnostic Center, University of Utah Health Science center, Salt Lake City, UT.

**Objective:** We studied 37 unrelated families with history of congenital adrenal hyperplasia (CAH) for 8 common mutations and gene deletion in the 21-hydroxylase CYP21 gene and found *de novo* mutations in the CYP21 gene in two CAH patients. **Methods:** Analysis for eight common mutations in the 21-hydroxylase gene as well as deletion of the entire gene was accomplished using polymerase chain reaction (PCR) followed by amplified created restriction site (ACRS) and southern blot followed by hybridization to a CYP21 specific probe. Linkage analysis was performed using microsatellite markers flanking the CYP21 gene. Moreover, 10 STP markers have been used to confirm the parentage in the two *de novo* mutation cases. **Results:** In two prenatal cases, the Intron 2 A->G mutation was identified in the proband, not in the fetus, although they had identical linkage markers. Subsequently, this mutation was confirmed absent in the parent's genome and misparentage has been ruled out. **Conclusion:** Our findings are consistent with the other studies with a frequency of *de novo* mutation of approximately 1.0 to 1.5% in the CYP21 gene. This high new mutation rate relative to the rate of 1 in 1 million for the other autosomal recessive disorders, must be considered and discussed in prenatal diagnosis and genetic counseling for this relatively common inherited disorder. The *de novo* mutation in the CYP21 gene is non-negligible.

**Expansion of fetal cells in the maternal circulation occurred using Progenitor RosetteSep<sup>TM</sup> enrichment procedure with IL-3, SCF and G-CSF.** A. Sharma<sup>1</sup>, H. Elicha Gussin<sup>1</sup>, D. Marquz-Do<sup>2</sup>, D. Martinez<sup>2</sup>, C. Horne<sup>2</sup>, D. Lewis<sup>2</sup>, R. Hoffman<sup>3</sup>, S. Elias<sup>1</sup>, J.L. Simpson<sup>2</sup>, F. Bischoff<sup>2</sup>. 1) Dept OB/GYN, UIC, Chicago, IL; 2) Dept OB/GYN and Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Dept Hematology/ Oncology, UIC, Chicago, IL.

Expansion of fetal cells in maternal blood could be an ideal method of non-invasive prenatal diagnosis, but its results have been inconsistent. We have continued this pursuit with the Progenitor RosetteSep<sup>TM</sup> cocktail (StemCell Technologies) to deplete unwanted lineage positive maternal blood cells (T cells, NK cells, thymocytes, myelomonocytic cells, neutrophils, macrophages, B cells and granulocytes). We investigated whether cells isolated from maternal blood could be expanded in vitro, and whether the expanded cells were of fetal origin.

**Methods:** Peripheral blood from 12 pregnant women in the first trimester of gestation was obtained. The RosetteSep<sup>TM</sup> cocktail was added to whole blood, which was then layered over Ficoll (1.077 g/ml) and centrifuged. The enriched progenitor cell fraction was placed in culture for expansion for 14 days, using three different conditions: **Condition A:** SCF, M-CSF, GM-CSF, G-CSF; **Condition B:** IL-3, SCF, G-CSF; **Condition C:** IL-3, SCF, G-CSF M-CSF, GM-CSF. Cells were analyzed by FISH using direct-labeled X- and Y- probes. **Results:** Expansion of cells occurred in 11 out of 12 subjects, under each of the three conditions. Samples from 5 women with male fetuses, and from one with a female fetus, were analyzed. FISH results predictably showed no Y in the cells from the subject with a female fetus. For subjects with male fetuses, male cells (range: 1-5 cells) were found in cultures in condition B in three specimens (gestational age 10 1/7 to 10 5/7 weeks); no male cells were found in conditions A or C for these same samples. One male cell was found in the A culture from another specimen (GA: 12 4/7 weeks).

Cells isolated from maternal blood using the Progenitor RosetteSep procedure can be expanded in vitro. Male cells were found in 4 of 5 samples from women carrying male fetuses, and optimal culture conditions for such fetal cells may differ based on gestational age.

**Investigation of a marker chromosome unmasks a de novo cryptic chromosome 22 rearrangement with 22q subtelomere and ARSA duplication.** *E. Mak-Tam, P.R. Wyatt, L. Grierson, D. Allingham-Hawkins, E. Capua, G. Johnson, J. Kennedy, N. Neill.* Dept Genetics, North York General Hosp, Toronto, ON, Canada.

We report unexpected FISH findings while investigating a satellited marker in an amniotic fluid. A healthy 34 year old female presented at the genetics clinic because of a positive integrated prenatal screen (IPS): elevated Beta hCG and slightly elevated AFP. The family history was negative. Fetal ultra sound showed an apparently normal fetus with mild right hydronephrosis. Fetal echocardiogram showed a normal fetal heart. Chromosome results on amniotic fluid was 47,XX,+mar in all cells examined. A chromosome 22p- variant was noted but was not seen in either parent. Parental karyotypes were normal. The marker was C-Band positive and appeared to be bisatellited. Various CEP and paint probes were used for marker identification. The marker was positive only for the alpha satellite 14/22 probe. No euchromatin was detected. Additional FISH workup detected cryptic abnormalities on the "normal" 22p- chromosome. The VYSIS DiGeorge/VCF probe showed two signals for the ARSA locus located on opposite ends of the chromosome. The CytoCell 22qTel probe also showed two signals similarly placed. We could not resolve if the extra signals were just below or above the centromere. The patient was counseled for risks associated with the de novo abnormalities of chromosome 22. Termination was performed out of country because of advanced gestation. The fetal X-ray was normal. The fetus (from photographs) appeared to have micrognathia, abnormal position of fetal fingers and a broad nasal bridge. The abnormal 22 in this case was a serendipitous finding and not detectable with routine G-Banding. It is difficult to determine how much testing is sufficient. However, in view of this case, if the origin of a marker is determined and a variant is noted on the chromosome of origin, further studies may be warranted to rule out cryptic changes.

**Fetal Cell Recovery from Whole Maternal Blood: A Comparison of Two Protocols.** *H. Stroh, B. Berlin, D.W. Bianchi, K.L. Johnson.* Division of Genetics, Department of Pediatrics, Tufts-New England Medical Center, Boston, MA.

**Objective:** Fetal cells are present in the maternal circulation although their low frequency inhibits their retrieval. We compared fetal cell recovery from maternal whole blood using a detachable immunomagnetic bead technique to that of a human progenitor cell enrichment cocktail.

**Methods:** Pregnant women identified as high risk (e.g. abnormal ultrasound or serum screening) or undergoing an invasive procedure were enrolled in the study. Blood samples were processed within 24 hours using a CD71 immunomagnetic detachable bead isolation procedure (Dynal, Inc.) (n=22) or the RosetteSep progenitor cell cocktail (Stem Cell Technologies, Inc.) (n=61) according to the manufacturers specifications. Male fetal cells were identified by FISH using X and Y chromosome-specific probes and/or real-time PCR amplification of DYS1. Fetal sex was confirmed by amniocentesis, plasma PCR, or delivery.

**Results:** Overall, the sensitivity of detection of fetal cells from women carrying male fetuses by FISH was comparable between the Dynal and RosetteSep protocols (30 and 24%, respectively), and higher for the detection of cells from aneuploid fetuses using the RosetteSep procedure (75% [3 of 4] versus 40% [2 of 5]). By PCR, the overall detection rate was 20% with no false positive results using Dynal, and 4% with one false positive result using RosetteSep. The RosetteSep protocol resulted in a higher total number of cells isolated.

**Conclusions:** The large number of maternal cells isolated using RosetteSep resulted in time-consuming manual FISH analysis, and PCR analysis was confounded by the high background of maternal DNA present. FISH following Dynal isolation was more rapid, and likely more amenable to automation. Although the detection rate of aneuploid cells was higher using RosetteSep, this represents a small number of aneuploid samples analyzed, and the overall sensitivity by both FISH and PCR favors the Dynal procedure. Taken together, these results suggests that the Dynal immunomagnetic detachable bead system should be refined and further explored in a larger number of subjects.

**Preimplantation genetic diagnosis (PGD) for couples at risk of X-linked disease and for balanced translocation carriers.** *S. Weremowicz<sup>1</sup>, C. Racowsky<sup>1</sup>, D.J. Sandstrom<sup>1</sup>, A. Nureddin<sup>1</sup>, K.V. Jackson<sup>1</sup>, L.E. Wilkins-Haug<sup>1</sup>, C.C. Morton<sup>1</sup>, P.M. Miron<sup>2</sup>.* 1) Brigham and Women's Hospital, Boston, MA; 2) UMass Memorial Medical Center, Worcester, MA.

Since July 2000, PGD has been offered at Brigham and Womens Hospital for couples at risk of X-linked disease and carriers of reciprocal translocations. FISH with differentially labeled probes was employed to identify the sex of the embryo or the unbalanced chromosome rearrangements. Prior to clinical application, probe validation was performed on 22 couples and controls. All couples from the X-linked disease group proceeded with PGD by interphase FISH on blastomeres biopsied from three day pre- embryos with probes specific to chromosome X (DXZ1) and chromosome Y (DYZ1). For 5/15 couples with the reciprocal translocation [t(Y;15)(q11.23;p11.1), t(4;13)(q33;q21.2), t(14;16)(q32.3;q11.2), t(1;18)(q42.1;p11.2), t(15;20)(p11.2;q11.2) probes were individualized and included D15Z4 (at 15cen), RB1 at 13q14, BAC14c2 at 13q31, as well as short and long arm specific telomere probes of chromosomes 16, 18 and 20. In four couples, aneuploidy for chromosome 21 was assessed with the LSI21 probe. All probes but one (BAC14c2) were from Vysis Inc. In the X-linked disease group 11 cycles resulted in 135 biopsied embryos; 42/135 were classified as XX. The remaining (n=93) were classified as XY, abnormal or uninterpretable. In total, 27 embryos were transferred in 11 cycles resulting in four pregnancies in 3/7 (43%) couples with deliveries of three healthy females and one twin pregnancy with no follow-up. For translocation carriers, 13 cycles were performed and 86 embryos were biopsied; 16/86 embryos (19%) were assessed as chromosomally normal or balanced. Fifteen embryos were transferred in eight cycles resulting in pregnancy in 3/5 (60%) couples. Three healthy babies were delivered and one pregnancy is ongoing. Although in our institution, PGD is considered a screening modality and not to supplant genetic amniocentesis or CVS, we believe it represents a critical aspect of a test menu for genetic disease, and that its availability provides an efficacious alternative.

**GENE TRANSFER OF SP-C PROMOTER DRIVEN HUMAN EXTRACELLULAR SUPEROXIDE DISMUTASE (hEC-SOD) PLASMID cDNA USING POLYETHYLENIMINE (PEI) CARRIER EXPRESSES THE PROTEIN IN LUNG EPITHELIUM OF ADULT MICE 7 DAYS AFTER TREATMENT.** *M. Ahmed, M. Whorton, N. Mason, R. Auten.* Dept Pediatrics/Neonatology, Duke Univ Medical Ctr, Durham, NC.

Rationale: Transgenic overexpression of hEC-SOD in mice protects against oxygen toxicity. Since oxygen toxicity may contribute to chronic lung disease of prematurity, gene therapy may target sufficient antioxidant protection to the most vulnerable compartment. Method: We mixed 2.6 mg PEI, a cationic polymer (av. MW=25kDa), with 2 mg hEC-SOD cDNA, at a nitrogen (PEI):phosphorus (cDNA) molar equivalent ratio = 10:1, and administered it once by aerosol to adult mice in 95% air 5% CO<sub>2</sub> (to increase minute ventilation) over 30 min. Mice were sacrificed at 1, 3, and 7 days after treatment, and lungs were inflation fixed for immunohistochemical detection of hEC-SOD expression using an affinity purified rabbit anti-hEC-SOD IgG. hEC-SOD transgenic mice and wild type mice were used as positive and negative controls. Results: Wild type mice showed no hEC-SOD expression, while hEC-SOD transgenic mice showed strong alveolar and bronchiolar expression. At 1 day after PEI-hEC-SOD treatment, there was strong alveolar and bronchiolar expression that persisted at reduced levels on days 3 and 7. Conclusion: PEI gene transfer of SP-C promoter driven hEC-SOD cDNA can provide hEC-SOD expression in alveolar and bronchiolar epithelium up to 7 days. Speculation: Gene therapy that leads to transient EC-SOD overexpression may protect the lung against oxidant stress of short duration.

**Maternal MTHFR Genotype is a Predisposing Factor for Nonsyndromic Cleft Lip/Palate.** *F. Desposito<sup>1</sup>, M. Schwalb<sup>1</sup>, M. Royle<sup>2</sup>, D. Streck<sup>1</sup>, J. Dermody<sup>1</sup>*. 1) Center for Human & Molecular Genetics, UMDNJ-NJ Medical School, Newark, NJ; 2) NJ State Department of Health and Senior Services, Trenton, NJ.

The thermolabile forms of MTHFR have been implicated in a variety of medical problems including coronary artery disease, spina bifida and complications of pregnancy. Previous studies of the correlation of this polymorphism in infants with CL/P have shown both positive and nonsignificant results (Mills, et al 1999; Shaw, et al 1998). We examined a group of affected children and their mothers gathered from a national CDC study of birth defects. DNA was extracted from buccal swabs from 17 non-Hispanic Caucasian mothers and their affected children diagnosed with either cleft lip, cleft palate or both. The patients were ascertained as part of the CDC's National Birth Defects Prevention Study. All of the specimens in this study were collected in New Jersey. Of the 17 samples available for testing, nine mothers of affected children with apparent non-syndromic CL/P had the homozygous 677T/677T genotype. Of the eight non-homozygous 677T/677T, three mothers were compound heterozygotes 677T/1298C. Four of the affected children also had the 677T/677T genotype. A control group of 18 had one person homozygous for 677T. The preliminary observation that nine of 17 (53%) mothers of infants ascertained with CL/P displayed the 677T/677T thermolabile form of MTHFR suggests that this maternal genotype may be a major predisposing factor in the risk of a child having nonsyndromic CL/P. Our control group showed a frequency of 5.5% consistent with a recent population study, which showed a frequency of 7.2% for this genotype in Caucasians (Esfahani, et al 2003). We are currently analyzing additional families, the possible role of maternal compound heterozygosity 677T/1298C, and implications for primary prevention by prenatal vitamin supplementation for women with homozygous MTHFR genotypes.

**HERMANSKY-PUDLAK IN PREGNANCY: A risk factor for obstetrical hemorrhage.** *S. Klugman, D. Barbouth, S.M. Carter, P. Dar, P.S. Bernstein, S.J. Gross.* Albert Einstein College of Medicine, Montefiore Medical Ctr, Bronx, NY.

**Background:** Hermansky-Pudlak syndrome (HPS) is an autosomal recessive disorder that presents with oculocutaneous albinism, bleeding diathesis and ceroid storage disease primarily affecting the lungs and gut. While HSP has been described in many ethnic groups, its prevalence is rare (1/500,000). However, among the Puerto Rican population, the prevalence rate is 1/1800. Molecular testing of the HPS1 gene is available specifically for those of northwest Puerto Rican descent and mutation analysis of the HPS3 gene is likewise available for patients of central Puerto Rican and Ashkenazi Jewish heritage. Mutations in the HPS1 gene have a detection rate of 97%. Pregnancy in patients with HPS has been rarely reported, with only two previous case reports that discuss pregnancy complications. We report another case of HPS in a pregnant Puerto Rican woman who has experienced significant bleeding in all three of her pregnancies.

**Case:** The proband, a 22 year old G3P1011, had an uncomplicated first pregnancy and was delivered of a term female infant by spontaneous vaginal delivery who was unaffected. The postpartum course was complicated by bleeding that required a platelet transfusion. Following this pregnancy, she had a significant episode of bleeding two weeks after an elective termination, which required platelet transfusion. The current pregnancy is with a different partner, who underwent molecular analysis for the HPS1 gene and was negative. At the time of publication, the patient was 35 weeks pregnant and has had an episode of epistaxis that required platelet transfusion.

**Conclusion:** Despite the general tendency towards increased risk of clotting during pregnancy, our patient exhibited bleeding complications common in HPS. Because these complications increase morbidity and potential mortality, pregnant patients with HPS require careful surveillance. Because of the high frequent carrier status in Puerto Rico, routine carrier testing should be considered in those of Puerto Rican descent.

**MSAFP screening from 21-24 weeks gestation: the Manitoba experience.** *N.K. Björklund<sup>1</sup>, K.M. MacDonald<sup>1,2</sup>, J.A. Evans<sup>1,2,3</sup>, B.N. Chodirker<sup>1,2</sup>.* 1) Biochemistry & Medical Genetics; 2) Pediatrics & Child Health; 3) Community Health Sciences; University of Manitoba, Winnipeg, Canada.

Most maternal serum screening programs do not interpret samples taken after 21 weeks gestation. The Manitoba policy has been to interpret the MSAFP for samples drawn before 25 weeks gestation. Risks for chromosomal anomalies are only interpreted up to 21 weeks. The purpose of this study is to review the Manitoba experience with MSAFP screening done at a late gestation defined as 21 weeks and <25 weeks. The Manitoba Maternal Serum Screening Program database was reviewed to determine descriptive statistics, including screen positive rate, for all 1st samples drawn at a late gestation (with correction of dates by ultrasound if available). Samples from twin pregnancies or with diabetes were excluded. Samples screened from 16 weeks to <17 weeks were used as a control group. We also reviewed all cases of neural tube defects (NTDs) identified with an MSAFP sample at 21-25 weeks to determine the sensitivity. 4870 patients (3.4% of the total) were screened at a late gestation over 13 years (1990-2002). Of these, 181 were elevated. The screen positive rate for the late samples was therefore 3.72% versus 2.49% for controls. The mean MOM for the late samples was 1.08 ( 0.67) compared to 1.03 ( 0.56). The medians were 1.19 and 1.12 respectively. While these differences are statistically significant ( $p < 0.001$ ), they are clinically unimportant. There were 8 late drawn routine screening tests associated with an NTD and 3 late samples after an anomaly was seen on ultrasound. 10 were elevated and one was normal. The one normal MSAFP was drawn at 23 weeks and spina bifida was diagnosed at birth (a "poorly covered" L5 defect). 7 of the 11 prenatally diagnosed cases of NTDs resulted in pregnancy terminations. Two cases of gastroschisis were also detected by late screening. Although MSAFP screening should ideally be done before 21 weeks gestation, we conclude that screening between 21 and 25 weeks gestation is feasible, with acceptable sensitivity and screen positive rates and late screening would still allow for intervention in affected pregnancies in many jurisdictions.

**Undetectable unconjugated estriol associated with Antley-Bixler syndrome: Further evidence for abnormal steroidogenesis.** *R.J. Hopkin<sup>1</sup>, N.P. Mulrooney<sup>2</sup>, S. Trumpy<sup>3</sup>*. 1) Dept Human Genetics, Cincinnati Children's Hosp Medical Ctr, Cincinnati, OH; 2) Dept Neonatology, Cincinnati Children's Hospital Medical Ctr, Cincinnati, OH; 3) Bethesda North Hospital Department of Obstetrics and Gynecology, Cincinnati OH.

Antley-Bixler syndrome (ABS) is a rare disorder characterized by radiohumeral synostosis, craniosynostosis with resultant midface hypoplasia, characteristic facial dysmorphism, bowing of the femurs, multiple joint contractures, and urogenital defects. Based on reports of affected siblings and affected children born to consanguineous couples ABS has been considered an autosomal recessive condition; however, other modes of inheritance have also been suggested. Genetic heterogeneity is likely. Several reports have invoked errors of steroid metabolism in the pathogenesis of ABS. Evidence for this has included reported association with maternal luteomas, fetal 21-hydroxylase deficiency, early pregnancy exposure to fluconazole, and lanosterol 14-alpha-demethylase deficiency. We report siblings with classic features of ABS. In the first pregnancy ultra sound documented facial dysmorphism, radiohumeral synostosis, ambiguous genitalia and femoral bowing. Examination after delivery confirmed the diagnosis of ABS. Chromosomes were normal, 46,XY. During the second pregnancy ultrasound including 4-D images again demonstrated findings suggestive of ABS. Amniocentesis revealed a normal karyotype 46,XX. Molecular studies of FGFR2 were negative. The diagnosis of ABS was confirmed following delivery. At 8 months 17-OH-progesterone, cortisol and ACTH response were normal. Maternal serum screening had demonstrated undetectable maternal serum unconjugated estriol (uE3) in each of the affected pregnancies implicating abnormal steroid metabolism in these children. Undetectable uE3 has been associated with a variety of errors in steroidogenesis: steroid sulfatase deficiency, congenital adrenal hyperplasia, and Smith-Lemli-Opitz syndrome, but to our knowledge has not been reported in association with ABS. This finding adds to the evidence that abnormal steroidogenesis plays an important role in the pathogenesis of this complex disorder.

**The influence of fetal loss on the presence of fetal cell microchimerism: a systematic review.** *D.W. Bianchi<sup>1</sup>, K. Khosrotehrani<sup>1</sup>, J. Lau<sup>2</sup>, D.H. Cha<sup>1</sup>, K. Johnson<sup>1</sup>*. 1) Division of Genetics, Department of Pediatrics, Tufts-New England Medical Ctr, Boston, MA; 2) Division of Clinical Care Research, Tufts-New England Medical Ctr, Boston, MA.

**Purpose.** Fetal cells enter the maternal circulation during most pregnancies but persist for years in only some women. We have previously shown that terminations of pregnancy result in a higher fetal cell transfusion into the maternal circulation (1). In addition, we reported, using DNA polymorphism analysis, that fetal microchimeric cells persisting in the liver of a woman originated from a miscarried fetus 19 years earlier (2). The objective of this study was to determine if pregnancy history influences the persistence of fetal microchimeric cells long after pregnancy.

**Methods.** We reviewed all published studies on fetal cell microchimerism, defined as the detection of male DNA in maternal tissue, that describe individual pregnancy histories, disease diagnoses and microchimerism status of their study subjects. The number of total pregnancies, births and sons, and history of fetal loss (spontaneous abortion and elective termination) were tested as factors potentially associated with the persistence of microchimeric cells.

**Results.** One hundred twenty-four subjects from eleven studies met the inclusion criteria. Only fetal loss was significantly associated with the presence of microchimerism (odds ratio 2.4, 95% confidence interval: 1.2-6.0).

**Conclusion.** These results suggest that fetomaternal cell trafficking following fetal loss may be important for the engraftment of microchimeric cells in maternal tissue. This may be due to an increased amount of fetomaternal transfusion or transfer of a cell type that is more likely to engraft. We recommend that future studies on microchimerism report detailed pregnancy information, as these data are critical for the understanding of factors that influence the development of fetal cell microchimerism.

1. *Am J Obstet Gynecol* 2001; 184:703-706.

2. *Hepatology* 2002; 36(5):1295-1297.

**Prenatal Diagnosis and Deletion Screening of Duchenne Muscular Dystrophy in Iranian Families.** *A. Tabarrokhi, R. Kariminejad, N. Almadani, H. Najmabadi.* Molecular Genetic , Kariminejads najmabadi genetic, Tehran, Tehran, Iran.

Duchenne Muscular Dystrophy (DMD) is one of the most common X-linked genetic disorders seen in children. Mutation in the DMD gene, coding for the protein called dystrophin, causes the severe muscle wasting disorders leading to wheelchair dependence by 10 to 12 years of age and progress relentlessly until death by the early 20s. In the absence of a cure, prenatal diagnosis (PND) appears to be the best approach to reduce the burden for these families. In the last 5 years, we have studied 80 families for deletion screening and linkage analysis. Initially three sets of Multiplex PCR were used for screening of 20 exons in the dystrophin gene. Followed by, three intragenic RFLPs (pERT 87-15/ BamHI, pERT87-8/TaqI, pERT 87-15/XmnI) and also two CA repeats (5- Dys MSA and 3 Dys MSA). Our findings indicate that, deletions were observed in 43 affected boys (approximately 54 percent). The most common deletions were found in exons 49, 50 and no deletion was detected in the Promoter (Pm) region. In 32 numbers of cases, the deletions were confined to the distal hot spot (exons 44-60), and in 11 numbers of cases deletions were related to the proximal hot spot (exons 4-17). In 53 families these three intragenic RFLPs were utilized and in 34 families one or more of these RFLPs were informative (64 percent). The most informative RFLPs in our population was BamHI (46 percent) and the least one was TaqI (28 percent). In 31 families Microsatellite repeat analysis was performed to identify the mutant alleles and in 12 families 5-DYS was informative and in one family 3-DYS was informative. Prenatal diagnosis was performed for 21 families (15 cases were C.V.S and 6 cases were Amnion). 13 fetuses had male sex which 11 cases were normal and two of which were affected. Also 8 fetuses had female sex which 3 of them were normal and 5 of which were carriers. So, multiplex PCR technology and three intragenic RFLPs, can be used effectively for PND and also carrier detection, but in Iranian families CA repeats 3- Dys MSA was found out that has minimum heterogeneity.

**Single cell co-amplification of microsatellite markers for preimplantation genetic diagnosis by haplotype analysis of Xq28 disease genes.** *N. Gigarel<sup>1</sup>, N. Achour-Frydman<sup>2</sup>, P. Burlet<sup>1</sup>, V. Kerbrat<sup>2</sup>, M. Vekemans<sup>1</sup>, R. Frydman<sup>2</sup>, A. Munnich<sup>1</sup>, P.F. Ray<sup>1</sup>.* 1) Dept Genetics, Hop. Necker Enfants Malades, Paris, France; 2) Dept Obs and Gynae, Hop. A.Beclere, Clamart, France.

Preimplantation Genetic Diagnosis first consisted in the selection of female embryos for patients at risk of transmitting X-linked recessive diseases. Advances in molecular biology now allow the specific diagnosis of almost any pathology. For families with an identified X-linked disease causing mutation, non-specific diagnosis by sex identification can be considered as sub-standard since it involves the unnecessary disposal of healthy male embryos and reduces success rate by diminishing the pool of embryos eligible for transfer. The most telomeric part of the X-chromosome long arm is a highly gene-rich region encompassing disease genes such as haemophilia A, X-linked adrenoleukodystrophy, X-linked hydrocephalus and incontinentia pigmenti (IP). To circumvent the risk of misdiagnosis due to a meiotic recombination between the gene of interest and the marker it is important to analyse at least two markers located on each side of the disease gene. We developed five single cell triplex amplification protocols with different combinations of microsatellite markers DXS1073, BGN, G6PD, DXS1108, DXS8087 and F8C. The choice of the set to use then depends on the diagnosed pathology and on the informativity of the markers for the concerned families. These tests will allow the diagnosis of all diseases previously mentioned providing that the genetic material of an index case allowing the identification of the morbid allele can be obtained. Amplification rates on single lymphocytes of the different markers ranged from 93-97% with allele dropout rates comprised between 0 and 19%. Familial linkage analysis was carried out for 10 couples. For each couple there was at least one of the multiplex primer set in which 2 markers were informative. So far PGD has been carried out for three patients at risk of transmitting X-linked adrenoleukodystrophy, X-linked hydrocephalus and hemophilia A. The latter is now undergoing a normal pregnancy.

**Validation of a test for aneuploidy in pre-embryo blastomeres at a USA national reference laboratory. A.**

*Anguiano<sup>1</sup>, V. Sulcova<sup>1</sup>, S. Ziebe<sup>2</sup>, I. Agerholm<sup>3</sup>, M. Bungum<sup>4</sup>, K. Erb<sup>5</sup>, K. Lyby<sup>6</sup>, C. Grondahl<sup>6</sup>, C.M. Strom<sup>1</sup>.* 1) Cytogenetics, Quest Diagnostics, Nichols Ins, San Juan Capistrano, CA; 2) Fertility Clinic, Rigshospitalet, Copenhagen, Denmark; 3) Fertility Clinic, Braedstrup Sygehus, Braedstrup, Denmark; 4) Fertility Clinic, Skive Hospital, Skive, Denmark; 5) Fertility Clinic, Odense University Hospital, Odense, Denmark; 6) Novo Nordisk A/S, Bagsvaerd, Denmark.

Preimplantation Genetic Diagnosis (PGD) for aneuploidy detection has been shown to increase IVF success rates in women of advanced maternal age. As PGD gains wider acceptance, it will be necessary to increase the number of laboratories capable of performing PGD with proficiency documentation. For assay validation, we tested blastomeres from 45 donated surplus pre-embryos that were obtained following informed consent from 4 centers in Denmark. No information was received on methods of cycle inductions nor medical history of donors. The pre-embryos were disaggregated, and individual blastomeres prepared and fixed on a coated glass slide. Commercially available probes for chromosomes 13, 16, 18, 21, 22 (MultiVysion PB, Vysis Inc.) were used. Additionally, a sequential hybridization for X and Y centromeres was performed. A control slide from metaphases from peripheral blood of a male individual was run with each assay. From morphological evaluation, 104 blastomere nuclei were expected. Giemsa staining demonstrated 97 nuclei (concordance 93%), and FISH results were informative in 93 (96%), partially informative in 2 (2%), and uninformative in 2 (2%). Of the 93 informative nuclei, 23 showed a diploid count for all tested chromosomes, 10 exhibited one chromosome aneuploidy, and 60 had more than one abnormality (of these, 6 were suggestive of triploidy). These results fulfill our requirements for assay validation and enable us to consider offering PGD for aneuploidy as part of our menu of FISH tests.

**Polar Body and Blastomere Analysis for Spinal Muscular Atrophy (SMA); SMN1 and SMN2 Exon 7 - 8 Deletion Detection Together With Single-Copy and Multi-Copy Flanking STR Markers.** *R.S. Ozen, S. Rechitsky, T. Sharapova, K. Laziuk, O. Verlinsky, A. Kuliev, Y. Verlinsky.* Reproductive Genetics Institute, Chicago, IL.

Spinal muscular atrophy (SMA) is an autosomal recessive disorder with a prevalence of 1/10,000 and a carrier frequency of 1 in 40-60 individuals. All types of SMA are caused by mutations in the survival motor neuron gene (SMN) locus mapped on chromosome 5q11.2-13. The SMN gene is present in two highly homologous copies, SMN1 and SMN2. Homozygous loss of functional survival motor neuron 1 (SMN1) alleles results in SMA, while homozygous absence of SMN2 gives no clinical phenotype. Since most SMA patients lack both copies of SMN1 exon 7, preimplantation genetic diagnosis (PGD) for SMA is based on the avoidance of SMN1 homozygous deletions, which is complex because of the sequence similarity between SMN1 and SMN2 genes, and requires simultaneous linkage analysis with STR markers. We performed PGD for SMA carriers, using polar body (PB) and blastomere analysis of exon 7 and exon 8 deletions in SMN1 and SMN2 genes together with closely linked STR markers. To test paternal SMN1 and SMN2 deletion patterns on each chromosome by blastomere analysis, single sperm testing was performed to establish the haplotype of normal and deleted alleles with multicopy STR marker D5S1556 (Ag1-CA), located on promoter region of both gene copies, and single copy STR markers D5S435, D5S351 and D5S610. We analyzed 49 PB and 36 blastomeres from 5 In Vitro Fertilization (IVF) cycles resulted in 4 ongoing pregnancies, two with confirmed twins and two with singletons (one confirmed by CVS and one is pending). The results show that the above system is reliable for PGD of SMA both by PB and blastomere analysis.

**Spectral karyotyping for use in preimplantation genetic diagnosis and detection of chromosome aneuploidy. R.**

*Owen<sup>1,2</sup>, J. Tong<sup>2</sup>, J. Ciselak<sup>3</sup>, Y. Verlinsky<sup>3</sup>, J.F. Weier<sup>2</sup>, R. Reijo<sup>2</sup>.* 1) University of California, San Francisco, Dept. of Pediatrics; 2) University of California, San Francisco, Dept. of Obstetrics, Gynecology and Reproductive Sciences; 3) Reproductive Genetics Institute, Chicago, IL.

Many couples enlist the help of in vitro fertility (IVF) clinics to aid in producing a successful pregnancy. Likewise, more women of advanced maternal age and families with a history of known genetic disorders are visiting IVF clinics to undergo preimplantation genetic diagnoses (PGD) to increase their probability of having a healthy child. Unfortunately, even with assisted reproductive technology and advances in molecular testing, the success rate is still low. Objective traits such as embryo quality, for example, do not always dictate the chance of pregnancy. Alternatively, chromosomal abnormalities may be a leading contributor to a significant proportion of unsuccessful pregnancies. Currently, screening for chromosomal abnormalities is performed by polar body or blastomere biopsy using interphase FISH analysis. However, commercially available kits used in screening can only detect the more common aneuploidies 13, 16, 18, 21, 22 and sex chromosomes. Here, we present spectral karyotyping (SKY) results used to determine its application to PGD in exploration of aneuploidy in single blastomeres. We have analyzed 39 metaphase spreads from 14 different couples hybridized with chromosome specific probes. Approximately 50% of these spreads were analyzable; those failed were due to lost chromosomal material or lack of chromosome spreading. None of those examined were identified as having a normal karyotype. Spectral karyotyping, in the future, with adequate technical expertise and optimal chromosome condensation and spreading may be able to predict aneuploidy and structural abnormalities, however, our results suggest it is not ready for clinical use.

**PREIMPLANTATION HLA TYPING.** *Y. Verlinsky, S. Rechitsky, O. Verlinsky, I. Tur-Kaspa, A. Horwitz, A. Kuliev.*  
Dept Molec Genet, Reproductive Genetic Inst, Chicago, IL.

We performed 378 cases of preimplantation genetic diagnosis (PGD) for 54 different conditions, including single gene defects, dynamic mutations and some medically relevant genetic variations. Unaffected embryos were pre-selected in 333 (88.1%) of them, resulting in 110 (33%) clinical unaffected pregnancies. The PGD protocols used provided an option not only for avoiding an affected pregnancy, but also for performing HLA typing to pre-select the HLA compatible donors for transplantation therapy in affected siblings. Single biopsied blastomeres were tested by multiplex nested PCR analysis to analyze simultaneously for mutations, linked markers, chromosomal aneuploidy and HLA genes, allowing the pre-selection and transfer back to patients only those unaffected embryos, which were HLA matched to the affected sibling requiring stem cell transplantation. The method was applied for the HLA genotyping in 24 cases in combination with PGD for thalassemia, Fanconi anemia, hyper immunoglobulin M syndrome, X-linked adrenoleukodystrophy and Wiscott-Aldrich syndrome, confirming the usefulness of preimplantation HLA matching as part of PGD. In addition, we performed 13 cases of preimplantation HLA typing without testing for causative gene, such as for different leukemias. This resulted in the embryo transfer of HLA matched embryos in 11 cycles, yielding 5 clinical pregnancies of which three have already resulted in the birth of HLA-matched healthy children to become potential HLA compatible donors for children requiring bone marrow transplantation. The data provide a realistic option for the couples desiring to establish a pregnancy potentially providing an HLA match progeny for treatment of affected siblings.

**Validation of Preimplantation Genetic Diagnosis for Clinical Applications.** *H. Tyson<sup>1</sup>, S. Chong<sup>1</sup>, A. Benner<sup>1</sup>, Z. Al Hasnan<sup>1</sup>, S. Katsanis<sup>1</sup>, K. Thrift<sup>2</sup>, A. Lawler<sup>2</sup>, G. Stetten<sup>2</sup>, Y. Zhao<sup>2</sup>, G.R. Cutting<sup>1</sup>.* 1) Inst Genet Med, JHU, Baltimore, MD; 2) Dept of OB/GYN, JHU, Baltimore, MD.

While prenatal diagnosis for single gene disorders using DNA from amniocytes or chorionic villus cells is performed almost exclusively in clinical laboratories with established quality assurance and quality control procedures, Preimplantation Genetic Diagnosis (PGD) is not widely available in the clinical setting. Since PGD offers a new testing option for couples at risk for single gene disorders, we have addressed this issue by validating a PGD test for the common autosomal genetic disorder cystic fibrosis (CF). All validation experiments were performed in a masked fashion and at least one wash blank was provided for every cell analyzed. To detect the CF mutation deltaF508, exon 10 of the *CFTR* gene was amplified by nested PCR of single lymphoblast cells heterozygous for deltaF508. Although 91% (61/67) of single lymphoblasts were amplified, the deltaF508 mutation was correctly identified in only 58% (34/59). Redesign of primers increased detection of deltaF508 to 98% (131/134), and wash blanks accompanying the lymphoblasts were not contaminated (0/38). While 86% (25/29) of de-identified discarded blastomeres were amplified for *CFTR* exon 10, 9% (6/67) of the accompanying wash blanks were contaminated. To address this issue, two washes were added to the blastomere biopsy procedure, reducing contamination to 2% (3/124). Since contamination remained, we incorporated a fluorescent polymorphic DNA marker (ID2) to detect non-fetal contaminating DNA. Reagent blank contamination was observed in 1/19 experiments which prompted a re-design of the protocol for assembling the PCR mastermix. Using the optimized PGD protocol, exon 10 and ID2 were amplified from 90% (18/20) of donated IVF blastomeres with no wash or reagent contamination detected (0/70). For couples with a mutation other than deltaF508, four previously published linkage markers flanking *CFTR* (D7S480, D7S486, D7S490, and D7S523) have been validated. We demonstrate that errors of single cell analysis can be detected and addressed by extensive validation thus allowing PGD test implementation in a clinical DNA diagnostic lab.

**A look at a minority population, primarily hispanic, in an urban prenatal diagnostic center: referral reasons, amniocentesis uptake, and abnormalities detected.** *C. Guze*<sup>1,2</sup>, *D. Baker*<sup>2</sup>, *R. Hassan*<sup>1</sup>. 1) Dept OB/GYN, King Drew Medical Ctr, Los Angeles, CA; 2) Dept Biology, California State Univ Dominguez Hills, Carson, CA.

**OBJECTIVE:** To describe the population referred to our inner city Los Angeles Prenatal Diagnostic Center (PDC) with a primarily Hispanic clientele. Specifically, we wanted to assess trends in referral reasons and amniocentesis uptake and to also assess the abnormalities found during the seven-year period (1995-2001). **METHODS:** All 3085 patients referred to the Genetics Unit for genetic counseling between January 1, 1995 and December 31, 2001 were evaluated through a retrospective review of records maintained by the unit. Patient records included race, age, referral reason, referral clinic, amniocentesis decision and results, and abnormalities. **RESULTS:** A total of 3085 patients received genetic counseling during the 7-year study period (1995-2001). The patient population consisted of 75.9% Hispanic, 22.4% African American and 1.7% other. The primary referrals were for AMA (48.3%) and MS-AFP (32.6%). The overall amniocentesis acceptance rate was 51.5%: AMA amniocentesis acceptance rate was 45.6% and the MS-AFP screen positive rate was significantly higher at 64.4%. There was a significant difference between the overall amniocentesis acceptance rate for Hispanics (47.7%) and African Americans (62.8%) and a significant trend downward of amniocentesis uptake between 1995 (63%) and 2001 (39%). This trend held for both ethnic groups. However, the acceptance rate for African Americans was consistently higher than the Hispanic acceptance rate by year and by referral reason. There were 151 (4.9%) abnormalities detected by ultrasound and amniocentesis among the 3085 patients referred. **CONCLUSIONS:** The acceptance of amniocentesis in the Hispanic and African American population in our PDC is significantly lower than what has been previously reported in the literature for Caucasians and the overall California statewide acceptance rate, and has significantly declined over the 1995-2001 time period. Supported by NIH MBRS R25 GM62252.

**In-vitro expansion of trophoblasts isolated from the maternal circulation: potential application in non-invasive prenatal diagnosis.** *E. Guetta, L. Gutstein-Abo, G. Barkai.* Danek Gertner Institute of Human Genetics, Sheba Medical Center, Tel-Hashomer, Israel.

The application of non-invasive prenatal diagnosis based on isolation of rare fetal cells from maternal blood will become a feasible alternative to the invasive procedures currently in use only if in-vitro expansion and subsequent metaphase chromosome analysis can be achieved. Trophoblasts originating from the placenta are present in the maternal circulation, albeit, at a frequency corresponding to a rare cell event. In an initial effort to develop a method that could potentially provide cycling cells for chromosome analysis, improve the efficiency of fetal cell detection and increase the cell yield, we studied the possibility of growing trophoblasts isolated from maternal blood in an in-vitro culture system. HLA-G, a trophoblast marker, aids in targeting these cells for application in non-invasive prenatal diagnosis. Thus, trophoblasts were isolated from maternal blood with magnetic activated cell sorting (MACS) and anti-HLA-G antibodies. The HLA-G positive fractions of cells were either directly analyzed or seeded in culture for 3-5 days. Fluorescent in-situ hybridization with sex chromosome probes was applied in order to identify fetal trophoblast cells in samples from women carrying a male fetus. The average number of fetal trophoblast cells expressing HLA-G increased 5-fold after culture ( $p < 0.01$ ). Accurate sex identification was accomplished in 93% of the samples when the in-vitro culture protocol was tested in a group of samples analyzed in the absence of prior knowledge of fetal sex. Improvement of this system may facilitate the development of a reliable, clinically applicable method for non-invasive prenatal diagnosis.

**Prenatal Ultrasound findings in Lissencephaly.** *S. Ghai<sup>1</sup>, K. Fong<sup>1</sup>, A. Toi<sup>1</sup>, S. Blaser<sup>2</sup>, A. Pai<sup>1</sup>, D. Chitayat<sup>1</sup>.* 1) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Canada; 2) Medical Imaging, Hospital for Sick Children, Toronto, Canada.

Lissencephaly is a neuronal migration disorder characterized by the absence of sulcation of the cerebral hemispheres. The condition is difficult to diagnose prenatally prior to 24 weeks gestation. Fetal ultrasound findings in 16 fetuses with lissencephaly who were followed prenatally at our institution were reviewed. Lissencephaly was confirmed by prenatal/postnatal MRI, and/or autopsy. In cases with Miller-Dieker syndrome (MDS), this was confirmed by FISH analysis for deletion 17p13.3. There were 6 cases with MDS and 10 cases with Walker-Warburg syndrome (WWS). Ultrasound examination was performed at 15-41 weeks gestation. Ultrasound images were reviewed for intra/extracranial findings with an emphasis on cerebral cortical development. The sylvian and parieto-occipital fissure, calcarine and ingulate sulcus and the sulci on the convexity of the brain were evaluated. One or more cerebral abnormalities were found in all fetuses. Ventriculomegaly was the most common finding and was present in 12 fetuses. In three cases it was isolated and in 9 cases it was associated with other findings. In 5 fetuses the initial findings were ventriculomegaly detected at 20 wks gestation or earlier. In the 11 remaining fetuses one or more findings of delayed cortical development were noted on ultrasound. The earliest gestational age lissencephaly could be suspected was 23 weeks. Other cranial findings included microcephaly (2), corpus callosum dysgenesis (1), absent septi pellucidi and cavum (2), Dandy-Walker malformation/variant (5), encephalocele (1), and lemon-shaped head (2). Extracranial findings included: eye and facial abnormalities (4), omphalocele (1), renal anomalies (1) and growth-restriction (3). The earliest and most common cranial abnormality which should raise the suspicion for possible lissencephaly is ventriculomegaly. Thus, follow-up ultrasound at 23-24 weeks in the third trimester with a detailed survey of the brain sulcation and gyration should be part of the investigation of mild ventriculomegaly. Fetal brain MRI as well as FISH for deletion 17p13.3 can assist in making the diagnosis.

**Invasive prenatal diagnosis in the Netherlands 1991-2000: a trend analysis in 117.430 pregnancies.** *N.J Leschot<sup>1</sup>, H.T.C. Nagel<sup>2</sup>, A.C. Knegt<sup>1</sup>, M.D. Kloosterman<sup>3</sup>, H.I.J. Wildschut<sup>4</sup>, F.P.H.A. Vandenbussche<sup>5</sup>.* 1) Dept. Clinical Genetics, Academic Medical Centre, Amsterdam, The Netherlands; 2) Dept. Obstetrics/Gynecology, Bronovo Hospital, The Hague, The Netherlands; 3) Dept. Obstetrics/Gynecology, Rijnstate Hospital, Arnhem, The Netherlands; 4) Dept. Obstetrics, Erasmus Medical Centre, Rotterdam, The Netherlands; 5) Dept. Obstetrics, Leiden University Medical Centre, The Netherlands.

Objective: The findings in the first 10 annual reports of the Dutch Working Party on Prenatal Diagnosis were combined and analysed for trends. In the Netherlands the same (standard) indications are used in all 13 centres. The annual number of births is about 200.000. There is no official screening program for Down syndrome and/or neural tube defects. Methods: In each annual report detailed data on all prenatal diagnostic procedures are listed. Follow up data about continuation or termination of pregnancy (TOP) for each indication are available for the last 5 years. Findings: The proportion of women that chose for prenatal diagnosis, about 5-6%, was rather stable, despite an increase in the total number of pregnant women of 36 years or older from 15.140 in 1991 to 25.730 in 2000! In a total of 117.430 pregnancies maternal age was the indication in 72%. The proportion of women of 36 years or older that chose for prenatal diagnosis was stable up to 1994 (47%) and then decreased to 34% in 2000. The reason is that, despite the absence of an official screening program, an increasing number of women with this indication asks for maternal serum screening or for nuchal translucency measurement. The total number of abnormal laboratory findings increased from 362 in 1991 to 638 in 2000. In 71% the parents chose for TOP. For the years 1996-2000: 88% of all trisomy 21 pregnancies (n=989) were terminated, for pregnancies with sex chromosome abnormalities (n=386) 54% were terminated. The mean annual number of TOP's was about 400, this is about 2% of all annual abortions in our country. The proportion of chorionic villi samplings decreased from 39%(1991) to 27%(2000).

**Prenatal Diagnosis of Hydrocephalus due to congenital stenosis of aqueduct of Sylvius (HSAS) by fetal MRI.** A. Kennedy<sup>1,2</sup>, J. Carey<sup>3</sup>, R. Hulinsky<sup>1</sup>. 1) OB Diagnostic Center, Univ of UT Hospital, Salt Lake City, UT; 2) Radiology, Univ of UT Hospital, Salt Lake City, UT; 3) Pediatrics, Univ of UT, Salt Lake City, UT.

Hydrocephalus is a common birth defect detected easily by ultrasound. X-linked hydrocephalus is the most common form of genetic hydrocephalus accounting for approximately 5% of aqueductal stenosis related hydrocephalus. Hydrocephalus due to congenital stenosis of aqueduct of Sylvius (HSAS; MIM #307000) is characterized by hydrocephalus, adducted thumbs, spasticity, agenesis of the corpus callosum, and mental retardation. HSAS along with MASA and X-linked spastic paraplegia type 1 are associated with mutations in the L1CAM gene. We report a 30-year-old G3 P2002 female who presented for prenatal care at 34 weeks gestation. Severe hydrocephalus was noted by ultrasound with cranial biometry out of chart range. Visualization of cranial contents was further compromised by skull ossification. MRI was requested for further evaluation specifically to differentiate severe hydrocephalus from hydranencephaly as this would influence mode of delivery (C-section contraindicated with hydranencephaly). Fetal MRI showed severe hydrocephalus and normal cerebellum with intact vermis. Visualization of fetal hands by ultrasound was difficult, however by MRI bilateral adducted thumbs were noted, highly suggestive of X-linked aqueductal stenosis as the etiology. Upon taking a detailed family history the mother reported a son from a previous relationship that has CP. Upon review of the child's CT and MRI, it was confirmed that he had agenesis of the corpus callosum. Due to family history, presence of adducted thumbs, and severe hydrocephalus the fetus was determined to have HSAS possibly due to a mutation in the L1CAM gene. The patient presented at 37 weeks gestation in labor and the child was delivered via C-section due to fetal head size. Postnatal CT scan confirmed aqueductal stenosis. Postnatal exam confirmed presence of bilateral adducted thumbs. Correlation between prenatal findings and postnatal outcome will be demonstrated using images from ultrasound, MRI, and postnatal exam. This case illustrates the usefulness of fetal MRI in conjunction with ultrasound to determine antenatal diagnosis.

**Simultaneous determination of fetal trisomies 18 and 21 by real-time quantitative PCR.** *W. Holzgreve<sup>1</sup>, B. Zimmermann<sup>1</sup>, L. Levett<sup>2</sup>, S. Hahn<sup>1</sup>.* 1) Dept OB/GYN, Univ Basel, Basel, Switzerland; 2) The Doctors Laboratory, London, UK.

The detection of gross chromosomal abnormalities is a major focus of prenatal diagnostics, of which the most common cytogenetic anomaly in live births is trisomy 21, also known as Down's Syndrome. Currently, prenatal diagnosis of genetic anomalies relies on invasive procedures such as amniocentesis and chorionic villus sampling (CVS), from which the full fetal karyotype is usually determined using cultured cells. The two week period needed for cultivation and subsequent analysis has proven to be associated with considerable parental anxiety. This two-week delay is also associated with considerable medical problems in those situations requiring therapeutic intervention. In order to address these needs, more rapid methods for the prenatal diagnosis of fetal chromosomal aneuploidies have recently been developed and implemented, such as multi-colour fluorescence in-situ hybridisation (FISH) as well as quantitative fluorescent PCR analysis of short tandem repeats (STRs). With the advent of real-time PCR it is now possible to measure nucleic acid concentrations with an accuracy that was not deemed possible only a few years ago. Examples are the analysis of gene expression or gene duplications / losses, where two-fold differences in nucleic acid concentration have routinely been determined with almost 100% accuracy. As our primary interest is in prenatal diagnosis, we have investigated whether real-time PCR could be used for the diagnosis of chromosomal anomalies, in particular the aneuploidies such as trisomy 21, where the difference in copy number is only 50%. The feasibility of such an approach was first tested in a pilot study, wherein we were able to demonstrate that trisomy 21 samples could be detected with 100% specificity and sensitivity. We have now modified this test to permit the simultaneous analysis of trisomies 18 and 21 and have in a large scale analysis demonstrated that our approach can be used for the highly reproducible and robust detection of only 1.5 fold differences in gene copy number. Our studies have also underscored that several criteria need to be met such as template preparation and primer purity.

**Is preterm delivery a risk factor for autistic disorders?** *N. Luscri, T. Stroud, J. Miles, N. Takahashi.* Dept Child Health, Div Med Gen, Univ Missouri Hospital, Columbia, MO.

Prematurity is a major risk factor of illness & disability. This study attempts to determine if prematurity is a risk factor in autistic disorders. This may assist clinicians in earlier diagnosis allowing a more favorable prognosis. Methods: Gestational age was available for 402/422 patients, all of whom met DSM-IV diagnostic criteria for autistic disorder, PDD-NOS or Aspergers. The average DOB was 1989, range 1943-2001. Preterm delivery was defined as gestational age <37 weeks, & very preterm delivery <32 weeks. The rates of preterm delivery for 402 probands were compared to Missouri population rates in 1991 (11.4% preterm; 1.9% very preterm). Phenotypic subgroups were analyzed individually to determine if prematurity predisposes to different outcomes or occurred in specific subgroups. Results: For the entire group, 12.9% (52/402) were preterm, & 2% (8/402) were very preterm. Children with physical or neurologic risk features (dysmorphism, brain abnormality, abnormal EEG, microcephaly, seizures), type of onset (regressive), and different autism behavioral phenotypes (typical vs mild social, communication or repetitive behavior impairments) and IQ/DQ subgroups were analyzed individually. There was no significant increase or decrease in preterm delivery rates for any of these subgroups. When analyzing patients for prenatal and perinatal complications, high levels of complications correlated with higher rates of preterm delivery: high prenatal complications [ $<37$  wk = 42.9% (6/14),  $p=.02$  &  $<32$  wk = 28.6% (4/14),  $p<.0001$ ]; high perinatal complications [ $<37$  wk = 50% (11/22),  $p<.0001$  &  $<32$  wk = 22.7% (5/22)  $p<.0001$ ]. Other potential risk factors such as socioeconomic status, maternal age, prenatal infection, APGAR scores, genetic syndromes, and other chromosome abnormalities were analyzed. Only low APGAR scores showed a significant correlation with prematurity. Conclusion: Autism is an etiologically and clinically heterogeneous disorder. Prematurity does not, however, occur more frequently in any of our recognized autism subgroups, in the classical autism diagnostic categories or in groups with severe vs mild symptoms in the three phenotypic domains.

**Fetomaternal hemorrhage identified through high MS-AFP and resulting in a brain infarct.** *D. Myles Reid<sup>1</sup>, J. Kingdom<sup>2</sup>, H. Sroka<sup>1</sup>, S. Blaser<sup>3</sup>, A. Toi<sup>4</sup>, D. Chitayat<sup>1</sup>*. 1) Medical Genetics, Mount Sinai Hospital, Toronto, ON, Canada; 2) Department of Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, ON, Canada; 3) Department of Pediatrics, Division of Medical Imaging, The Hospital for Sick Children, Toronto, ON, Canada; 4) Department of Medical Imaging, Mount Sinai Hospital, Toronto, ON, Canada.

We report a case of elevated second trimester maternal serum alpha-fetoprotein (AFP) associated with fetomaternal hemorrhage which resulted in fetal brain infarct. The patient was a 28-year-old primigravida woman of Colombian descent, who was referred following a high MS-AFP of 16.05 MoM detected on MSS done at 16.6 weeks gestation. The uE3 was 1.14 MoM and the hCG was 3.09 MoM. Detailed fetal ultrasound performed at 18.2 weeks gestation demonstrated normal fetal anatomy, excluding a fetal structural abnormality as the cause of the raised MS-AFP. In addition, Doppler studies were normal suggesting normal placental function. MSS repeated at 18.4 weeks gestation showed increase in the MS-AFP of 162 MoM and high hCG of 5.54 MoM. Maternal abdominal ultrasound as well as total body MRI showed no evidence of a tumor. A repeat MS-AFP at 19 weeks gestation was 170 MoM. Fetal MRI performed at 20 weeks gestation did not detect any abnormalities to account for the elevated AFP levels. Betke-Kleihauer test was completed and the results were consistent with an estimated fetomaternal transfusion of 25ml. Since the fetal-placental blood volume at this gestation is approximately 150ml/kg the estimated fetal blood loss was 25%. In view of these findings we repeated the fetal MRI which showed bilateral cystic changes in the region of the middle cerebral artery. In addition, the posterior parietal and occipital lobes were thinner than expected suggesting cell loss in these regions. The fetal head growth was also lagging. The appearance was suggestive of an in utero ischemic event within both middle cerebral artery territories. It is likely that the fetus experienced a profound hypoperfusion episode at the time of the fetomaternal hemorrhage.

**The prenatal molecular karyotype: DNA microarray analysis of cell-free fetal DNA (cffDNA) in amniotic fluid.**

*P.B. Larrabee<sup>1</sup>, E.S. LeShane<sup>1</sup>, E. Pestova<sup>2</sup>, K. Wilber<sup>2</sup>, U. Tantravahi<sup>3</sup>, J.M. Cowan<sup>1</sup>, K.L. Johnson<sup>1</sup>, D.W. Bianchi<sup>1</sup>.*

1) Division of Genetics, Department of Pediatrics, Tufts-New England Medical Center, Boston, MA; 2) Vysis, Inc., Downers Grove, IL; 3) Department of Pathology, Women and Infants' Hospital, Providence, RI.

**Background:** Prenatal cytogenetic diagnosis traditionally consists of metaphase karyotype analysis of cells obtained by amniocentesis or CVS. We have previously shown that large quantities of cffDNA are present and easily amplified in amniotic fluid (Clin Chem 2001;47:1867-9). The use of cffDNA may allow for more rapid and detailed molecular analysis of fetal genetic material, thus enhancing the current standard of care. **Methods:** With IRB approval, 6 frozen amniotic fluid supernatant samples were obtained from mid-trimester pregnant women carrying 4 male and 2 female fetuses. After thawing and, in some cases, removing residual intact cells, cffDNA was extracted, labeled, and hybridized to the GenoSensor™ Array 300 (Vysis, Inc.) This microarray allows for the quantitative analysis of DNA sequences from all human chromosomes, including subtelomeres. All analyses were performed blindly without knowledge of fetal karyotype. **Results:** Samples from all 4 male fetuses showed increased hybridization signals on SRY and decreased hybridization signals on DXS580, DMD, KAL, STS, AR, DXS7132, XIST, and OCRL1 when compared to female reference DNA. Samples from both female fetuses showed the reverse when compared to male reference DNA. Because these markers had an overall analytical sensitivity of 90% and specificity of > 99%, the gender of every specimen tested was therefore identified correctly. Work is in progress on the evaluation of abnormal samples confirmed by karyotype, including whole chromosome gains such as trisomy 21. **Conclusions:** The preliminary results suggest that microarray technology can be applied to cffDNA extracted from amniotic fluid. Correct identification of fetal gender was achieved in all cases. The use of DNA microarrays for prenatal genetic analysis of cffDNA in amniotic fluid may allow for rapid screening of amniotic fluid samples and the enhanced diagnostic capabilities of a molecular approach.

**The logistics of integrated prenatal screening for Down syndrome in Ontario, Canada.** *T. Huang, I. Lasis, C. Meier, A. Summers.* Genetics Prog, North York Hosp, Toronto, ON, Canada.

Although second trimester triple marker screening is effective in detecting Down syndrome (DS), there has been concern over its high false positive rate (FPR) of 7%. The Integrated Prenatal Screening (IPS) is expected to reduce the FPR to 1.5% while improving the detection rate (DR) from 72% to 85%. IPS is carried out in two steps. The first step, between 10 and 14 weeks of gestation, involves an ultrasound for nuchal translucency (NT) and a blood sample for pregnancy-associated plasma protein A (PAPP-A). The second blood sample, taken between 15 and 18 weeks of gestation, is for alpha-fetoprotein, unconjugated estriol, and human chorionic gonadotrophin. The final result is based on the integration of the two parts. IPS was introduced at North York General Hospital (NYGH) in November 1999. IPS was first offered by geneticists and genetic counsellors at NYGH, and then expanded to physicians of patients delivering at NYGH, and finally to health care providers (HCP) within our region. Prior to the introduction of IPS, a screening protocol, patient brochure, counselling guideline and requisition were developed. Education sessions and rounds were organized to educate HCP at NYGH and in the region. Sonographer specific medians based on 50 NT images were established for each ultrasonographer before they were enrolled in the programme. A three-page requisition was used for the IPS. The first page is for NT first and then PAPP-A, the second page is for the second blood sample; the last page is for the patient's chart. Patients are advised to have the NT measured prior to the first blood sample, and then return for the second blood sample at an appropriate gestation. All screen positive women were counselled in the Genetics Program at NYGH. To date, ~15,000 women have been screened using IPS. The drop out rate for IPS is ~5%. The number of sonographers enrolled in the IPS programme has increased from 3 to 133. Initially, limited availability of NT was a problem, but now the vast majority of patients have NT measured. IPS has been successfully introduced into our practice, it has performed as expected and currently comprises 50% of our screening volume.

**Inter-laboratory comparison of fetal DNA detection from common maternal plasma samples using real-time PCR.** *K.L. Johnson, D.W. Bianchi, S. Elias, W. Holzgreve, J.L. Simpson, K.W. Klinger.* NICHD Fetal Cell Isolation Study (NIFTY).

**Background:** Analysis of cell-free fetal (cff) DNA from maternal plasma offers great potential for non-invasive prenatal genetic diagnosis. To further evaluate the clinical utility of this assay, we developed and validated a standard protocol to determine if cffDNA sequences could be reproducibly amplified and measured. We then compared its performance across multiple laboratories using a common set of specimens. **Methods:** Each of five participating centers collected 20 mL of peripheral blood from 20 pregnant women (10-20 weeks). The plasma fraction was separated according to a common protocol, divided, and frozen in five aliquots. One aliquot was shipped to each participating laboratory, where DNA was extracted according to a standard protocol. All plasma samples were then analyzed blindly for the presence and quantity of total DNA (GAPDH) and male fetal DNA (SRY) by real-time PCR. Genomic DNA was isolated from female and male cells at one center, quantified, and shipped to the others to serve as GAPDH and SRY standards, respectively. **Results:** Efficiency of amplification of known quantities of DNA was consistent amongst all centers. The mean quantity of male DNA amplified from maternal plasma when the fetus was male ranged from 51.14 to 228.20 genome equivalents (GE)/mL. The sensitivity of the assay for detection of male DNA when the fetus was male varied from 31.4 to and 97.1% among centers. Centers that extracted and amplified more DNA per mL of maternal plasma had superior sensitivities of Y chromosome sequence detection. A total of four false positive results were obtained, yielding more consistent specificity (92.8 to 100%). **Conclusions:** All centers were able to consistently amplify standard DNA that was frozen and had been shipped considerable distances. This demonstrates that DNA is a stable analyte and that the PCR technique used here is reliable and reproducible. Differences in sensitivity demonstrate that for assay reproducibility, emphasis should be placed on development of a robust and thoroughly optimized protocol for the extraction of DNA from maternal plasma to provide for a clinically relevant analytical tool.

**Development of highly sensitive allele-specific PCR based allelic discrimination method for non-invasive prenatal detection of cystic fibrosis mutations.** *O. Nasis, G. Sathe, L. Jackson, T. Otevrel.* Center for Molecular Diagnostics and Genome Research, Department of Obstetrics and Gynecology, Drexel University College of Medicine, Philadelphia, PA 19102.

Cystic Fibrosis is a genetic disease caused by mutations in the membrane conductivity gene, CFTR. Many individuals are non-affected carriers of this disease and can pass the defective gene to their children. If both parents are carriers of the mutated gene, a high probability exists that the child will inherit both mutated copies and will be affected with cystic fibrosis. In such high-risk cases, it is important to conduct prenatal screening. The current prenatal diagnostic tests are highly reliable; however, they are invasive techniques that carry a risk of fetal loss. To reduce the need for these procedures, our efforts are focused on the development of non-invasive prenatal screening methods using maternal blood samples. Fetal cell-free DNA is known to be present in maternal circulation during pregnancy, but its extremely low quantity and the overwhelming amount of maternal DNA causes difficulty in fetal genetic analysis. Our approach employs an allele specific PCR technology that has been improved to enable allelic discrimination and detection of mutations in samples containing 200 - 20,000 fold excess of wild type gene sequences. The procedure has been developed and tested for cystic fibrosis mutations deltaF508 and D1152H. The method would be applicable in situations when both parents of the fetus are carriers of the cystic fibrosis mutations, but have a defect in different parts of the CFTR gene. In this case, the presence or absence of paternal mutation can be determined in DNA isolated from blood of the pregnant woman. Exclusion of the paternal gene defect would then eliminate the need for an invasive prenatal diagnostic procedure.

**Cell-Free Fetal DNA Levels in Maternal Plasma before and after Elective First Trimester Termination of Pregnancy.** *T. Wataganara*<sup>1</sup>, *A.Y. Chen*<sup>2</sup>, *E.S. LeShane*<sup>1</sup>, *L.M. Sullivan*<sup>3</sup>, *L. Borgatta*<sup>2</sup>, *D.W. Bianchi*<sup>1</sup>, *K.L. Johnson*<sup>1</sup>.  
1) Division of Genetics, Departments of Pediatrics, Obstetrics and Gynecology, Tufts-New England Medical Ctr; 2) Departments of Obstetrics and Gynecology, and; 3) Departments of Mathematics and Statistics, Boston University Medical Ctr, Boston, MA.

**Objective.** Quantitation of fetomaternal hemorrhage (FMH) may be useful in certain clinical situations. Several methods have been investigated to measure FMH, but none has proven to be reliable. This has led to debate about the presence/extent of FMH. We investigated levels of cell-free fetal DNA (cfDNA) in maternal plasma before and after elective surgical and medical first trimester termination of pregnancy (TOP) to explore the possibility of using this analyte as a novel marker of FMH. **Methods.** With IRB approval, pre and post-procedure blood samples were drawn from 71 and 63 pregnant women undergoing surgical and medical TOP, respectively. DNA was extracted from plasma by standard protocols. Real-time quantitative PCR was used to measure the levels of *DYS1* as a marker of cfDNA, and  $\alpha$ -globin as a marker of total DNA. Post-termination levels were adjusted based on the half-life of cfDNA in maternal blood. Fetal gender was confirmed by analysis of placental tissue. **Results.** cfDNA was detected in gender unknown pre-termination samples from 27 (38%) and 29 (46%) of the subjects in the surgical and medical arms, respectively, as early as 32 days of gestation. cfDNA increased 4.2 genome equivalents/mL/week. In the surgical arm, the sensitivity of pre-procedure cfDNA detection was 92.6% (27 of 29 known male fetuses). The mean level of adjusted post-termination cfDNA was higher than expected based on the anticipated increase in cfDNA due to advancing gestation (paired t test,  $P=0.0017$ ). In the medical arm, 6 patients had increased cfDNA levels for up to 11 days following TOP. **Conclusions.** cfDNA can be reliably quantified in early first trimester maternal plasma. The elevation of cfDNA that occurs shortly after surgical TOP may reflect FMH or destruction of trophoblastic villi, and may remain elevated for several days following medical TOP. These data suggest that cfDNA is a sensitive and reliable marker to quantify FMH.

**Identifying fetal cells in maternal circulation by FISH - Feasibility for prenatal diagnosis.** *S. Shimizu, M. Sherwood, R. Wapner, L. Jackson.* Department of Obstetrics and Gynecology, Drexel University College of Medicine, Philadelphia, PA.

Fetal cells in maternal circulation are a potential source of non-invasive prenatal genetic diagnosis. Hamada et al (1993) and Krabchi et al (1999, 2001) quantified male fetal cells in a few maternal blood samples predominantly at 18 to 22 wks gestation using no cell separation or enrichment and counting cells with a Y fluorescence *in situ* hybridization (FISH) signal. To further assess this approach for prenatal diagnosis in a larger series at earlier gestation, we obtained blood samples from pregnant women between 10 and 16 wks gestation. Consecutive unselected maternal blood samples were processed to yield 16 male and 16 female fetus containing pregnancies. 2 ml of maternal blood was processed without enrichment by simple hypotonic pretreatment and Carnoy fixation of all nuclei. FISH was performed, and fetal XY cells were identified and counted in a background of maternal XX cells. Both manual and automated fluorescent microscopic scanning was performed. All 16 male pregnancies yielded XY positive cells by FISH. The number of such cells varied between 1.5 and 10 per ml of maternal blood. No XY positive cells were found in 16 female pregnancies. Automated microscope scanning of fewer samples found similar results. Higher numbers of cells were found in a small number of samples of pregnancies carrying fetuses with various chromosomal aneuploidies. This study confirms and extends the findings of Hamada and Krabchi that there is a small but consistent population of fetal cells present in maternal blood samples (95% c.i. 94-100% of pregnancies). The type and origin of these cells cannot be determined from this study as the method removes potentially identifying cytoplasmic characteristics. This study further demonstrates that these cells are present in late first trimester at a time when early prenatal diagnosis is desired. The method is inadequate for any real clinical application but indicates that sufficient cells are present in a small maternal blood volume for further development of a clinically useful diagnostic approach using these cells.

**Prenatal Diagnosis of Mosaic Trisomy 14: A Case Report and Literature Review.** *E. Wood, K. Blakemore, G. Stetten.* Johns Hopkins University, Baltimore, MD.

Trisomy 14 mosaicism is associated with a distinct phenotype which was established based on fifteen cases ascertained postnatally due to mental and physical anomalies. This phenotype includes growth and mental retardation, dysmorphic facial features, congenital heart defects, and abnormal genitalia in males. Tissues showing the trisomy 14 cell line include blood and skin. Fives cases of prenatally diagnosed trisomy 14 mosaicism have been published, ascertained by amniocentesis for advanced maternal age. Two of these five cases were terminated; one after a sonogram revealed hydrocephaly, and one which was found to have low-set ears, transverse palmar creases, clinodactyly, clubfeet, and an abnormal hip at autopsy. In three of the five cases, the pregnancy was continued. All three infants were reported as grossly normal at the time of birth, although long-term follow-up is not available. Additional tissue studies on these three cases, including in utero fetal blood, and blood and skin samples after delivery revealed normal chromosomes with no evidence of trisomy 14. The present case was ascertained when amniocentesis for advanced maternal age in a 41 year old, para 1011 Caucasian woman, revealed 2/17 colonies to be trisomic for chromosome 14. These colonies were on two separate coverslips. A second amniocentesis and an intrahepatic fetal vein blood sampling revealed a normal female karyotype in 21 colonies and 126 cells, respectively. UPD studies showed normal, biparental inheritance of chromosome 14. A sonogram and fetal echocardiogram at 20 weeks gestation revealed no fetal anomalies. Follow-up studies after delivery showed a 46,XX karyotype in 100 cells from a placental biopsy and 100 cells from cultured cord blood. This child, now 3 weeks old, is non-dysmorphic and showing normal development. This case serves as another example of the difference in phenotype when trisomy 14 mosaicism is diagnosed prenatally versus postnatally.

**Oculoauriculofrontonasal Syndrome (OAFNS): Early Prenatal Diagnosis and Autopsy Findings.** *J. Pierre-Louis<sup>1</sup>, J. Johnson<sup>2, 3</sup>, S. Keating<sup>4</sup>, D. Chitayat<sup>1, 2</sup>.* 1) Prenatal Diagnosis & Medical Genetics Program; 2) Depts. of Obstetrics & Gynecology; 3) Medical Imaging; 4) and Laboratory Medicine & Pathobiology, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada.

Oculoauriculofrontonasal dysplasia (OAFNS) is a rare developmental field defect with findings of both frontonasal dysplasia sequence and oculoauriculovertebral spectrum. It represents an abnormal morphogenesis of the frontonasal eminence. A total of 23 cases have been reported and the etiology is unknown. We report the prenatal ultrasound and autopsy findings in a case. A 46-year old, G3P1SA1L1 woman of British descent presented with a pregnancy conceived through donor intra-uterine insemination. An ultrasound at 12.4 weeks gestation for nuchal translucency screening showed an abnormal profile with retrognathia and suspected cleft lip and palate. The nuchal translucency measured 1.6mm and the nasal bone was absent. The patient was counseled and decided to have CVS that showed a normal female karyotype. A 3-D ultrasound showed an extensive craniofacial abnormality including severe micrognathia, a large facial cleft extending from the upper lip to the inner canthus of the right eye and back to the pharynx. There was a second less severe cleft on the left side, severe hypertelorism, low set ears and an abnormal nose. The couple decided to terminate the pregnancy. The autopsy confirmed the prenatal findings consistent with oculoauriculofrontonasal syndrome including bilateral cleft lip and palate, absent auricle and ear canal on the right, hypoplastic low-set and posteriorly rotated auricle and obliterated ear canal on the left, absent eyelid on the right and fused eyelids on the left, notched broad nasal tip, ocular hypertelorism and severe micrognathia. OAFNS has been described in the literature, but to the best of our knowledge this case is the first early diagnosis of this severe condition. In this instance, 3D ultrasound provided critical information enabling the couple to make an informed decision with respect to the management of the pregnancy. It also illustrates the ability to evaluation the fetal profile in details at the time of the 11-14 week scan.

**False negative interphase FISH assay on three prenatal cases of trisomy 18.** *C. Yu<sup>1</sup>, J. Bofill<sup>2</sup>*. 1) Dept Preventive Medicine; 2) Dept Obstetrics and Gynecology, University of Mississippi Med Ctr, Jackson, MS.

Prenatal interphase FISH assay is a powerful tool for determining the common aneuploid, and is becoming increasingly demanded in the prenatal diagnostic laboratory. However, when only limited numbers of nuclei are analyzed, the results of the prenatal interphase FISH study should be interpreted with caution. We report here three cases with false negative FISH studies and reemphasize the importance of conventional cytogenetic analysis. **Case 1.** KC, a 21-year-old black female at 26-weeks-gestation, referred for diagnosis due to polyhydramnios. Interphase FISH showed a male fetus with normal number 13,18 and 21. Cytogenetic study revealed a 46,XY,der(13)t(13;18)(p11.2;q11.2) karyotype. **Case 2.** BJ, a 21-year-old black female at 20-weeks-gestation, referred for diagnosis due to multiple fetal anomalies. Interphase FISH showed a male fetus with normal number 13, 18 and 21. Cytogenetic study indicated a 46,XY,i(18)(q10) karyotype. **Case 3.** IL, a 38-year-old white female at 34-weeks-gestation, referred for amniocentesis because of AMA and a fetal heart anomaly. Interphase FISH showed a female fetus with normal number 13, 18 and 21. Cytogenetic study revealed a 47,XX,+18cen- karyotype. In case 1, the derivative chromosome 13 contained no 18 centromere, in case 2, the isochromosome 18 contained only a single 18 centromere, and in case 3, one of the 18 centromeres had only a weak fluorescent signal that was missed during the process of image capturing. Prenatal interphase FISH study is a rapid and precious method for aneuploid detection, however, there are also pitfalls. The FISH analysis must be performed extremely carefully, and with a critical eye - especially when the information leads to clinical decisions without complete traditional cytogenetic analysis.

**Application of comparative genomic hybridization using microarrays to routine prenatal diagnosis.** *B.L. Shaffer<sup>1</sup>, P.D. Cotter<sup>2</sup>, A.B. Caughey<sup>1</sup>, M.E. Norton<sup>1</sup>.* 1) Ob/Gyn and RS, Univ Calif San Francisco, San Francisco, CA; 2) Children's Hosp Oakland, Oakland CA.

Complete genomic hybridization using microarrays (aCGH) has great potential for application in clinical genetics, including prenatal diagnosis. Compared to standard cytogenetics, aCGH provides increased resolution, more rapid results and potentially lower cost. We evaluated the utility of aCGH if applied to routine prenatal karyotyping by reviewing results from the UCSF Prenatal Diagnosis Program from 1984-2000 (n=45,833). We retrospectively determined which abnormal results would have been detected or missed using the UCSF HumArray, which includes 2464 clones. Using population frequencies, we also estimated the number of additional abnormalities not detected by routine karyotyping that would have been identified by aCGH. 1351 karyotype abnormalities were present (2.9%). 829 (62%) were trisomies and monosomies detectable by aCGH. 61 (4.5%) other unbalanced abnormalities would also have been identified as abnormal (9 deletions, 6 duplications, 36 unbalanced rearrangements and 10 triploidies (69,XXY). 209 balanced rearrangements, 10 triploidies (69,XXX), and 2 tetraploidies (92,XXXX) (16%) would not have been detected. Of 240 (18%) mosaic results (76 amniocentesis, 164 CVS), an uncertain percentage would have been identified. In a population of 45,833 patients, there would be approximately 94 clinically significant microdeletions, including cryptic subtelomere abnormalities, identifiable via aCGH. Use of aCGH for prenatal diagnosis in this population would have resulted in detection of 890 of the 902 cases (98.7%) of unbalanced abnormalities of definite phenotypic significance to the fetus, missing 12 cases of triploidy or tetraploidy and 209 balanced rearrangements. Of the 240 mosaic results, 164 were CVS samples, with most due to confined placental mosaicism. aCGH provides some clear advantages, detecting the majority of phenotypically significant abnormalities as well as many not identified with current technology. The trade off is primarily in missing abnormalities of lesser or unknown significance to the fetus, such as mosaics and balanced translocations.

**Cavum Veli Interpositi: Prenatal diagnosis and long term follow up.** *P. Shah<sup>1</sup>, S. Blaser<sup>2</sup>, A. Toi<sup>3</sup>, R. Babul-Hirji<sup>4</sup>, K. Chong<sup>4,5</sup>, D. Chitayat<sup>4,5</sup>.* 1) Department of Pediatrics, Mount Sinai Hospital, Toronto, ON, Canada; 2) Department of Pediatrics, Division of Medical Imaging, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; 3) Department of Medical Imaging, Mount Sinai Hospital, The University of Toronto, Toronto, Ontario, Canada; 4) Department of Pediatrics, Division of Clinical and Metabolic Genetics, The Hospital For Sick Children, University of Toronto, Toronto, Ontario, Canada; 5) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada.

**Objective:** To describe the prenatal findings and the postnatal outcome in two cases diagnosed with cavum villi interpositi. **Case 1:** Diagnosis of cavum villi interpositi of 3.5 x 3.0 x 1.2 size was made on routine prenatal screening at 33 weeks in non-consanguineous parents. There was no history of prenatal exposure to infection, drugs or toxins. No dysmorphic features were identified at birth. The absolute size of the cyst did not change during follow up MRI at 1 year of age, however relative size decreased. There was normal increase in the head circumference. Neurodevelopmental assessment revealed normal milestones at 2 years of age. **Case 2:** Diagnosis of cavum villi interpositi 3.2 X 1.1 X 1.7 size was made on a routine prenatal screening at 33.5 weeks gestation. The parents were consanguineous and their previous child died of lethal multiple congenital malformations. There was no history of prenatal infection, drugs, or toxins. No dysmorphic features were noted at birth. Serial ultrasonography at monthly intervals revealed minimal decrease in size. MRI at 9 months of age revealed unchanged CVI but dilated cavum septum pellucidum and cavum vergae. The rise in head circumference was within normal range. Neurodevelopmental examination at 11 months was age appropriate. **Conclusions:** Cavum villi interpositi is a rare finding on prenatal ultrasound screening. Despite the appearance of a large intracerebral cystic structure on pre and postnatal cranial imaging, CVI is associated with normal head growth and development.

**A novel approach for enrichment of fetal cells using a microfabricated silicon membrane.** *C. Somprasit<sup>1</sup>, D.A. Marquez-Do<sup>1</sup>, D. Yamanishi<sup>2</sup>, J.L. Simpson<sup>1</sup>, F.Z. Bischoff<sup>1</sup>.* 1) Dept OB/GYN, Baylor College of Medicine, Houston, TX; 2) AVIVA Biosciences, San Diego, CA.

Fetal cells in maternal blood have long been sought as a source of DNA for non-invasive genetic testing, either for direct analysis of fetal cells (aneuploidy) or for use as a complementary or additive screening test concurrent with ultrasound and maternal serum analyte screening. However, a consistently reliable enrichment technique resulting in optimal recovery of intact fetal cells has not yet been developed. Major obstacles have been cell loss during enrichment and lack of fetal specific antibodies. **OBJECTIVE:** To evaluate an alternative method of fetal cell enrichment. **DESIGN:** To investigate the feasibility of fetal cell isolation from maternal blood using a newly developed Fetal Enrichment Kit (AVIVA Bioscience). **METHODS:** Following IRB approval, peripheral blood (10 cc) from four women carrying a male fetus at 10 to 29 weeks gestation was obtained and processed using proprietary reagents. Briefly, specimens were processed within 2-24 hours using AVIwash solution and AVIPrep in order to aggregate and separate maternal RBCs. Magnetic AVI Beads coated with antibody cocktail were added to remove WBCs following one hour incubation. The recovered fraction was then enriched using a microfabricated silicon membrane to separate cells based on size (slot width of 2-4 microns). Final enriched cell population was spread onto a glass slide, air dried, and then fixed in Carnoy's solution. Using standard FISH methods, nuclei were scored for presence of the X (labeled with spectrum green) and Y (labeled with spectrum orange) chromosome-specific signals. Fetal male cells were identified on the basis of one signal for X and one signal for Y. **RESULTS:** In this pilot study, at least one fetal (XY) male cell was detected in each case with a range of 715 to 5886 total cells recovered. **CONCLUSION:** This novel enrichment approach offers promising potential in improving fetal cell isolation through use of a microfabricated silicon membrane to separate cells based on size. Further studies to compare sensitivity and specificity with alternative MACS and non-MACS based methods are ongoing.

**Quantitative detection of fetal DNA using dried maternal blood spots for generalized prenatal screening.** *J.L. Simpson<sup>1</sup>, D. Dang<sup>1</sup>, C. Somprasit<sup>1</sup>, D.I. Martinez<sup>1</sup>, D.E. Lewis<sup>2</sup>, F.Z. Bischoff<sup>1</sup>.* 1) Dept OB/GYN; 2) Dept of Immunology, Baylor Col Medicine, Houston, TX.

The accuracy and reliability of Realtime PCR to detect cell-free fetal DNA sequences in maternal plasma has been proven. However, generalized clinical application requires more effective methods for collection of large numbers of patient samples as well as protocols that minimize the potentially damaging effects of anticoagulant, storage and shipping conditions need to be incorporated. **OBJECTIVE:** Prospective examination of the efficiency of fetal DNA detection using dried maternal blood spots and Realtime PCR. **METHODS:** Prior to an invasive prenatal procedure, blood (3-5 drops) from a finger prick was placed on filter paper for each of 70 women (mean gestational age 12.6 weeks, range from 11.1 weeks to 35 weeks). Filters were coded and DNA analysis performed in a blinded study. DNA from blood spots was extracted using the QIAamp DNA blood kit. Presence of fetal Y-chromosome (DYS1) and the ubiquitous autosomal GAPDH gene sequences were measured with the TaqMan assay. **RESULTS:** In 40 of 48 (83%) cases confirmed to have a male fetus, Y-chromosome specific sequences were detected. Fetal Y-specific sequences detected ranged from 0 to 49.5 (mean 10.79) Geq/ml blood compared to 17465 to 340188 (mean 129960) Geq/ml GAPDH. In the 5 of 22 (33%) female cases in which Y-sequences were detected, mean (2.8 Geq/ml) amount detected was significantly less ( $P < 0.0003$ ) than the male cases. **CONCLUSION:** Fetal DNA detection using dried maternal blood spots is reliable, and appropriate cut-off values for fetal sequence detection can be identified. Blood spot analysis will provide a simple method for transport and collection, enabling cell free fetal DNA to be incorporated into noninvasive screening regimes (maternal serum analytes, fetal nuchal translucency).

**Prenatal diagnosis of Turner Syndrome in association with Dandy-Walker malformation.** *M. Ben-Yishay, S. Klugman, P. Dar, M. Bebbington, S.J. Gross.* Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, New York.

**Background:** The Dandy Walker syndrome includes cerebellar vermis dysgenesis and cystic dilation of the fourth ventricle. The syndrome has been associated with chromosomal disorders, single gene disorders and environmental exposures. In more than 80% of cases, additional anomalies can be demonstrated. The association of the syndrome with the 45 X monosomy (Turner Syndrome) has been rarely reported and only one case diagnosed prenatally.

**Case:** We report a 31 year old primigravida with a negative family history who had abnormal findings on second trimester ultrasound. She had had an uncomplicated prenatal course including normal maternal serum screening. The sonographic findings included an absence of the cerebellar vermis, hypoplasia of the cerebellar hemispheres and a thickened nuchal fold. The rest of the exam including the ventricles was normal. The patient underwent amniocentesis which revealed 45X by fluorescent in-situ hybridization (FISH) and karyotyping.

**Conclusion:** This case highlights the relationship between Dandy-Walker malformation and Turner syndrome. Detailed scanning of the posterior fossa should be included in the prenatal evaluation of a fetus with monosomy X prior to genetic counseling as this finding can be subtle on sonography, yet have substantial consequence with respect to prognosis.

**Dandy-Walker variant and 47,XXY.** *G.A. Jervis<sup>1</sup>, J.L. Angel<sup>2</sup>, B.G. Kousseff<sup>1</sup>*. 1) Regional Genetics Program, Univ South Florida, Tampa, FL; 2) Florida Perinatal Associates, Tampa, FL.

Dandy-Walker malformation is characterized by (1) hydrocephalus, (2) cyst in posterior fossa and (3) defect in cerebellar vermis through which the cyst communicates with the 4th ventricle. Dandy-Walker variant (DMV) shows smaller 4th ventricle cyst, less vermian hypoplasia and less severe or absent hydrocephalus. Dandy-Walker malformation/variant can be part of Mendelian disorders, including Meckel-Gruber and Walker-Warburg syndromes, of chromosomal abnormalities, including trisomies 13,18,21 and Turner syndrome or due to teratogens, including alcohol, Coumadin and maternal diabetes. Klinefelter syndrome is the most common sex chromosome abnormality with an incidence of 1.18 per 1,000 births. Clinical features include postpubertal small atrophic testes, small penis, gynecomastia, tall stature and dull mentality. Twenty percent of men with Klinefelter syndrome have major malformations with no clear pattern, including cleft palate, unilateral renal agenesis, microcephaly, and omphalocele (Gorlin, *Syndromes of the Head and Neck*, 4th Edition, 2001, p 62). We present a 33-year-old Caucasian woman, G4P0030, three first trimester spontaneous abortions, with an elevated MSAFP of 2.66 MoM, resulting in a 1 in 213 ONTD risk. Prenatal sonogram at 17 weeks showed an 8.9 cm cystic mass in the posterior fossa that communicated with the 4th ventricle at the level of the inferior cerebellar vermis, Dandy-Walker variant. Amniocentesis revealed 47,XXY. Termination was performed at 19 weeks. We were able to find one case report of isolated mild ventriculomegaly and Klinefelter syndrome, one case report of a fatal cerebral AV malformation in Klinefelter syndrome and several reports of seizures and essential tremors in men with Klinefelter syndrome. The DMV and Klinefelter syndrome in our patient could be coincidental, since both conditions are relatively common. However, this case emphasizes the importance of offering chromosome analysis whenever fetal sonogram reveals CNS anomalies and suggests that DMV may be a feature of Klinefelter syndrome.

**Prenatal diagnosis dilemma: Fetus with del(5)(p13.1p14.2) diagnosed postnatally with Cornelia de Lange syndrome.** *R. Hulinsky<sup>1</sup>, H. Winesette<sup>2</sup>, K.M. Dent<sup>3</sup>, R. Silver<sup>4</sup>, J. King<sup>3</sup>, A. Lowichik<sup>2</sup>, Z. Chen<sup>3</sup>, D.H. Viskochil<sup>3</sup>.* 1) OB Diagnostic Center, Univ of Utah, Salt Lake City, UT; 2) Dept of Pathology, Univ of Utah, Salt Lake City, UT; 3) Dept of Pediatrics, Univ of Utah, Salt Lake City, UT; 4) Dept of Obstetrics/Gynecology, Univ of Utah, Salt Lake City, UT.

We report a 28-year-old G2 P1 referred for abnormal ultrasound findings. Targeted ultrasound at 20.4 weeks revealed diaphragmatic hernia, single umbilical artery, nuchal skin thickening and abnormal upper extremities. The patient's mother reported six first trimester miscarriages and a stillborn infant. The couple was counseled regarding aneuploidy as well as chromosome translocations due to the maternal history. Amniocentesis revealed a karyotype of 46,XY,del(5)(p13.1p14.2). Parental chromosomes were normal. Oligohydramnios and IUGR presented in the third trimester. The couple was counseled extensively regarding poor prognosis, specifically in the context of a chromosome deletion, oligohydramnios, and diaphragmatic hernia. The couple was undecided about delivery and postnatal management plans. Precipitous delivery at 36.4 weeks gestation resulted in a liveborn infant who died at 2 hours of life. Autopsy revealed a male infant with physical characteristics of Cornelia de Lange syndrome (CdLS). The infant had the facial gestalt of CdLS, a left-sided diaphragmatic hernia, and oligodactyly of the upper limbs. Additional findings included a cleft in the soft palate, hypoplastic kidneys, short feet, VSD, hirsutism, and hypospadias.

This case illustrates the difficulty of prenatal counseling in the context of a previously uncharacterized chromosome anomaly. Prenatal diagnosis of CdLS based on sonographic findings has been reported, yet no genetic etiology has been identified. While studies have indicated an association with partial trisomy 3q, none have been reported in association with deletion 5p. In this case, the chromosome abnormality obscured full clinical assessment of the differential diagnosis of diaphragmatic hernia with limb defects and subsequent counseling, which should include CdLS. It points out one of the difficulties inherent in prenatal diagnosis.

**Prenatal diagnosis and autopsy findings in Catel-Manzke syndrome.** *R. Teitelbaum<sup>1</sup>, L. McLeod<sup>2</sup>, A. Pai<sup>1</sup>, S. Keating<sup>3</sup>, A. Toi<sup>4</sup>, L. Velsher<sup>5</sup>, D. Chitayat<sup>1, 2</sup>.* 1) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, University of Toronto, Toronto, Canada; 2) Department of Obstetrics and Gynecology, Division of Maternal Fetal Medicine, Mount Sinai Hospital, University of Toronto, Toronto, Canada; 3) Department of Laboratory Medicine and Pathobiology, Mount Sinai Hospital, University of Toronto, Toronto, Canada; 4) Department of Medical Imaging, Mount Sinai Hospital, University of Toronto, Canada; 5) Department of Medical Genetics, North York General Hospital, Toronto, Canada.

Catel-Manzke syndrome is an inherited condition characterized by micrognathia, cleft palate, glossoptosis, cardiac abnormalities and an accessory (usually triangular) bone at the base of the index finger (hyperphalangism).

We report a case of Catel-Manzke syndrome detected prenatally in a female fetus. A 31-year-old healthy primigravida woman of Anglo Saxon descent was referred to our center at 20 weeks gestation with multiple congenital abnormalities.

The fetal ultrasound showed Tetralogy of Fallot, micrognathia, abnormal hand posturing, clinodactyly, talipes, and abnormal toe spacing. The fetal karyotype was normal, and FISH for deletion 22q11.2 was negative.

The autopsy confirmed the prenatal ultrasound findings including Robin sequence with micrognathia, U-shaped cleft palate, and low set ears. There was tetralogy of Fallot, persistent left superior vena cava, over-riding aorta, ventricular septal defect, and bicuspid pulmonic valve. There were bilateral talipes and a wide sandal gap. The left hand had a widened space between the third and fourth fingers. There was fifth finger clinodactyly with hypoplastic distal phalanges bilaterally.

To the best of our knowledge, this is the second case of Catel-Manzke syndrome detected prenatally. The inheritance of the condition was initially thought to be x-linked. However, the few females reported with this condition raises the possibility of an autosomal recessive or genetically heterogeneous condition.

Program Nr: 2547 from 2003 ASHG Annual Meeting

**Detecting disease associations using htSNPs.** *J.D. Cooper, J.M. Chapman, J.A. Todd, D.G. Clayton.* JDRF/WT Diabetes and Inflammation Laboratory, Cambridge Institute of Medical Research, Wellcome Trust/ MRC Building, Addenbrooke's Hospital, Cambridge, CB2 2XY.

Within small genomic regions in European populations haplotype diversity can be limited and typing all known polymorphisms may lead to considerable redundancy. Previously, our laboratory suggested that linkage disequilibrium (LD) and haplotype diversity within such regions can be captured by a much smaller subset of markers, which we termed "haplotype tag SNPs" (htSNPs). We argued that typing htSNPs could potentially capture most of the information for association between a trait and one or more causal variants. Subsequently, a number of optimal methods for choosing htSNPs have emerged. However, little attention has been given to how such studies should be designed and analysed. We propose: (1) step-up, step-down and exhaustive subset algorithms to select htSNPs, based on measuring the ability of htSNPs to predict allele frequencies at another locus using an  $R^2$  measure; (2) a two-stage procedure for genotyping htSNPs; (3) a new "multi-locus TDT" to test for association between a trait and htSNPs due to LD with one or more causal variants.

Program Nr: 2548 from 2003 ASHG Annual Meeting

**High-throughput genotyping database system to manage multivariate phenotypes in complex trait analysis.** *S.E. Fiddy, R.F. Mott.* Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom OX3 7BN.

Successful genetic dissection of complex traits requires handling of considerably more data than had previously been anticipated. Sample sizes of tens of thousands of individuals have been collected in some linkage study designs and genome wide association strategies are likely to need hundreds of thousands of single nucleotide polymorphisms. Managing such large datasets and reducing error to hitherto unobtainable levels can be best achieved with a high degree of automation. We have developed a relationship database system that copes with these requirements by using the Microsoft .NET framework and SQL Server 2000. The system is accessed using a web front-end for cross platform usage. As a result the system is cost-effective and easy to distribute to other users. Flexibility is a key feature enabling a variety of genotypic markers to be handled, including SNPs and microsatellites, collected from a variety of genotyping platforms. For example, the system can automatically upload genotypes from the Sequenom mass spectrometer and can quality check the genotypes using Mendelian inheritance checking. Stored data can be extracted into various file formats for further analysis using standard genetic software. A major strength of the system design is ability to amalgamate a number of phenotypes and covariates for same subject treating them as a single entity. This makes it possible to implement easily multivariate genetic analysis of complex traits.

**Grid computing in genetics: concepts, schedulers, and preliminary experiences.** *M.M. Barmada, R.E. Evans, T.J. Bates.* Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA.

It is increasingly becoming evident that statistical genetics requires enormous computing resources to successfully address issues such as empirical p-value estimation, complete multipoint linkage analysis, haplotype frequency estimation, and linkage disequilibrium mapping. Statistical methodologies involving coalescent theory and Markov-Chain Monte-Carlo (MCMC) based simulation methods require long runs and are highly amenable to parallel processing strategies. We present here our preliminary experiences with the development of distributed grid-based computing systems to decrease the computational time required for successful application of these techniques. This work is not intended to be an overarching review of all possible mechanisms for grid computing, but rather to introduce the concept and some of its advantages and disadvantages, and to present a system which has been of significant use at our institution. Reasons for the adoption of grid-based architectures (as opposed to the more traditional symmetric multiprocessor (SMP)-based or parallel (MPI/PVM-based) architectures) will be discussed. We evaluated two common grid-enabling open-source technologies for their performance in a statistical genetics environment: MOSIX (now OpenMosix), and Suns GridEngine. For our hardware architecture, under common (and exaggerated) usage loads the performance of GridEngine enabled systems far outranked that of OpenMosix based systems on the same hardware architecture. Other metrics used to compare the two systems included ease of setup, ease of reconfiguration (a common occurrence), and implementation across heterogeneous hardware architectures. These experiences have identified several key features of grid design and new distributed computing technologies which we feel will be of utmost importance for statistical genetics.

**On comparison of tree-based methods and neural networks using genetic data.** *C.H. Chen<sup>1</sup>, C.L. Chen<sup>1</sup>, C.J. Chang<sup>2</sup>, C.S.J. Fann<sup>1</sup>.* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Department of Medical Research, National Taiwan University, Hospital, Taipei, Taiwan.

Data mining methodologies, such as tree-based methods and neural networks, were being used to facilitate gene mapping for complex traits. Researchers often apply these methods, by related phenotypic and genotypic covariates, to subdivide the study sample into more homogenous subgroups. In general, analyzing homogenous subgroup may increase power to detect linkage, comparing with that for the whole sample in the presence of heterogeneity. However, when dealing with smaller sample, it is unclear about the impact to the power of this approach because homogeneous subgroup also means that only fewer samples are available in the subsequent analysis. In corporation of non-parametric linkage analysis, we explore this question by empirical comparison of classification and regression tree methods and multi-layer neural networks using GAW12 simulation Data. In addition, to test the accuracy of the above classification rules, train-and-test, cross-validation, and bootstrap methods are used to estimate error rates for large, intermediate, and small sample size, respectively. Our result shows that, under some conditions, the performance of classification and regression tree methods and Neural Networks are similar in samples of large sizes.

Program Nr: 2551 from 2003 ASHG Annual Meeting

**Using Multivariate Association Modeling Algorithms to Characterize Relationships in Multivariate Data.** *C.C. Abney, R.A. Miller, D.T. Burke, N.J. Schork.* Dept. of Psychiatry, UCSD School of Medicine, La Jolla, CA.

Often researchers collect information on more than one phenotype, clinical outcome, biochemical measure, etc. with it in mind that some of these variables are likely related. Determining which subset of a larger group of variables is associated with, e.g., alleles at a particular genetic locus, is of considerable interest. We have developed a novel methodology to achieve this. This methodology leverages information about both differences in the mean (or average) level of a set of phenotypes across, e.g., genotypic categories, as well as differences in the relationships those variables might have across those categories (i.e., covariances). It essentially uses a multivariate likelihood ratio test that simultaneously assesses mean and covariance matrix differences among a set of variables. The proposed tests are therefore more comprehensive and powerful than many traditional multivariate tests which do not leverage information about both means and covariance matrices in their construction. We have developed software and a web-based interface for our methodology. We showcase the methodology on a large data set investigating the genetic basis of longevity and age-related parameters in a novel mouse cross. The proposed methodology is extremely flexible and provides new directions for QTL mapping in humans and model organisms.

Program Nr: 2552 from 2003 ASHG Annual Meeting

**PedigreeQuery - software for pedigree drawing.** A. Kirichenko. Laboratory of Genetic Analysis, Institute of Cytology and Gene, Novosibirsk, Russia.

Pedigree drawing is an important task in many studies in population genetics and epidemiology. It requires special software tools, because it is too difficult to do it manually especially if sample is very large and has intricate structure. We propose a new method of step-by-step pedigree drawing which was released in software PedigreeQuery. The method is based on expanding a pedigree by adding new nuclear families to already drawn pedigrees. This method allows drawing separate parts of pedigree, expanding in them into required direction. The principle of step-by-step pedigree drawing allows graphic representation of distribution of phenotypes in specific pedigrees and structure of kindred relations in studying sample. It also allows tracing paternal and maternal genome transfer and reconstructing an origin of rare alleles in a population. The software allows drawing pedigrees with a difficult structure, those containing consanguinity loops, individuals with multiple mates or several related families. Incoming data are data in LINKAGE format. Graphical presentation of pedigree is written into file in EPS format. Pedigrees are represented in standard form. There may be different subscribes under symbols. The symbols may be paint over according to the feature they are assigned to. The software has flexible setup system for graphic representation of the pedigree. The software, PedigreeQuery is freeware and may be downloaded from <http://mga.bionet.nsc.ru/PedigreeQuery/PedigreeQuery.html>.

Program Nr: 2553 from 2003 ASHG Annual Meeting

**Haplotype Inference in Nuclear Families.** *S. Lin, A. Chakravarti, D.J. Cutler.* Inst Medical Genetics, Johns Hopkins Univ, Baltimore, MD.

Phase information can be critical to the mapping of disease genes. From population genetics principles, it is posited that the sequences surrounding a relatively recent disease mutation but itself unchanged by recombinations and other mutations stretch on the order of several tens of kb. Acquiring accurate haplotypes on such lengths would increase the power to detect disease mutations in uncommon, complex diseases considerably. Since current statistical methods of haplotype reconstruction of individual genotypes have been found to be accurate only within regions of high LD (~10-20kb), genotypes from nuclear families are minimally necessary to achieve a high degree of accuracy beyond such lengths. Yet, even with such data, phase information is often not complete due to missing data and non-informative sites. To account for such ambiguities, we have constructed an algorithm which incorporates a probabilistic model based on a population genetic model (infinite allele sampling from a neutral population) within a Markov chain-Monte Carlo framework to reconstruct haplotypes within nuclear families. Application of the program on sequence data from eight loci spanning > 1 Mb on the human X chromosome, shows that the fraction of neighbor relations correctly called is greater than 93%. This algorithm is well suited for data sets with small families in which the haplotyping feature of Genehunter and other programs designed primarily for linkage analysis fails.

**Genetic Analysis of Function-Valued Traits.** *J. Zhao, M.M. Xiong.* Human Genetics Ctr, Univ Texas, Houston, Houston, TX.

A trait that varies as a function of a continuous variable such as age or time is referred to as a function-valued trait. Genetic analysis of function-valued traits will provide a general framework for longitudinal genetic studies that are a powerful tool for identifying complex genetic structure of common diseases and discovering strategies for prevention of diseases. However, genetic models of function-valued traits have not been well developed. In this report, we propose a general framework for genetic analysis of function-valued traits. In the proposed model, the genotypic values are decomposed into mean, additive and dominance effect functions. The developed models applied to population samples. We use two-step procedure to estimate the mean and genetic effect functions. We first use a mixed linear model to fit a longitudinal population sample data set. The mean and genetic effect functions are modeled as fixed effects. Correlation structure of repeat measurements is modeled as a random effect. The REML method is used to estimate the mean and genetic effect functions as well as variance components in the mixed linear model. Then, we use smoothing spline to smooth the raw estimators of the mean and genetic effect functions. We analytically show that the estimators of the genetic effect functions are asymptotically unbiased and converge to the true genetic effect functions in the single homogeneous population. We propose several hypotheses to test association of candidate locus with function-valued trait and to detect when the genetic factors start or cease to affect the trait. To avoid spurious association of the test in the presence of population substructure, we present a modified genetic model for function-valued traits. We analytically show that the test based on modified model is valid in the presence of population substructure. The methods are applied to simulation data to evaluate their performance and to illustrate their applications. The preliminary simulation results show that although the mathematical forms of the mean and genetic effect functions are unknown, the proposed methods can accurately estimate the mean and genetic effect functions.

**The Effect of Initial Allele Distributions on Linkage Measures in the Context of Study Design.** *F. Sievers, E.D. Kelly.* Hitachi Dublin Laboratory, Trinity College, Dublin, Dublin, Ireland.

Many bioinformatics analyses and simulations are hampered by an overly large parameter space and the difficulty of controlling these parameters during an experiment. We conduct a population simulation in which a large portion of the parameters (pedigree and the recombination pattern) is kept constant. The only parameters changed will be the alleles assigned to the founding individuals. We can therefore study the purely statistical effect of varying allele distributions while keeping familial and micro-biological effects constant. Hitachi's population generator is used to simulate extensive pedigrees comprising a large number of individuals and stretching over several generations. Off-spring are produced using rules mimicking mating and recombination patterns observed in nature. Individuals at the initial generation are each set up with two unique founder alleles that can be tracked unambiguously over time. We monitor the flow and distribution of founder alleles over time and determine the distribution of haplotype lengths identical by descent. In the second stage we assign concrete alleles to the founder alleles and compare the haplotype lengths identical by descent to the haplotype lengths identical by state. We re-assign alleles repeatedly and show the effect this has on various measures like Linkage Disequilibrium, Hardy Weinberg Equilibrium and (estimated) Haplotype Frequencies using Hitachi's Hitagene software suite. Allele re-assignment is done in two different ways: (i) alleles are simply re-shuffled, therefore preserving the (initial) allele distribution and (ii) alleles are assigned with different distributions. Fluctuations in the measures due to (ii) are not surprising but those due to (i) are investigated carefully. We also demonstrate that simulated populations in conjunction with repeated allele re-assignment can be useful in the process of study design.

**Genotype Data Merge Software.** *A. Presson*<sup>1</sup>, *E. Sobel*<sup>2</sup>, *K. Lange*<sup>1,2,3</sup>, *J.C. Papp*<sup>2</sup>. 1) Depts of Statistics; 2) Human Genetics; 3) Biomathematics, UCLA, Los Angeles, CA.

There is a growing need to merge data within and between genotyping centers and to merge current data with legacy data sets. The resulting larger sample sizes confer greater statistical power. Accurate merging is particularly important for successful association studies. However, manually merging data is time-consuming and error prone. The increasing frequency of collaboration between genotyping centers calls for automation in the interest of saving time and improving accuracy. Some factors that complicate merging include differences between genotyping hardware; binning methods; molecular weight standards; and curve fitting algorithms. The result is that genotypes from different sources do not differ by the same amount, both between and within markers. This makes it insufficient to align genotypes by adding a constant number of base pairs to the alleles of one of the data sets, even when considering individual markers. Other factors making it difficult to accurately merge data include few samples in common, and samples drawn from different ethnic groups, in which allele frequencies may vary.

We propose statistical algorithms to find the most probable correspondence between alleles by considering various aspects of the data. One method uses an algorithm that evaluates alignments by a chi-square homogeneity test (based on allele frequencies). Another method uses a Bayesian approach that chooses alignments with the greatest posterior probability. Additional procedures are necessary to incorporate information such as ethnicity, number of samples in common, data set size, and (if the user chooses) allele-lumping. When alleles with small frequencies are difficult to align with confidence, allele-lumping will allow the alleles to be combined into a single 'super allele'. We have developed software that will automate our genotype merging methods. The software evaluates the characteristics of the input data sets, allows user-specified merging options, and includes an error analysis to assign a merge-quality score. If there is too little information to confidently merge data sets, the software will recommend that they be analyzed separately.

**An ensemble method for gene discovery based on DNA microarray data.** *S.-Q. Rao<sup>1, 4</sup>, X. Li<sup>2, 3</sup>, T.-W. Zhang<sup>2</sup>, Z. Guo<sup>2, 3</sup>, K.L. Moser<sup>5</sup>, E.J. Topol<sup>1, 4</sup>, Q. Wang<sup>1, 4</sup>.* 1) Center for Cardiovascular Genetics, Dept. of Cardiovascular Medicine, the Cleveland Clinic Foundation, Cleveland, OH; 2) Dept. of Computer Science, Harbin Institute of Technology, Harbin, China; 3) Dept. of Biomedical Engineering, Biomathematics and Bioinformatics, Harbin Medical University, Harbin, China; 4) Dept. of Molecular Cardiology, the Cleveland Clinic Foundation, Cleveland, OH; 5) Dept. of Medicine, Institute of Human Genetics, University of Minnesota, MN.

The advent of the microarray technology has promised to provide new insights into the complex biological systems by monitoring activities of thousands of genes simultaneously. Current analyses of microarray data focus on classification of biological types, for example, tumor versus normal tissues. A future scientific challenge is to extract disease-relevant genes from the bewildering amount of raw data. This is one of the most critical themes in the post-genomic era, but it is generally ignored due to the lack of an efficient approach. In this paper, we developed a novel recursive-partition-tree-based ensemble method for gene extraction that can be tailored to fulfill multiple biological tasks including (1) precise classification of biological types; (2) disease gene mining; and (3) target-driven gene networking. We also gave a numerical application for (1) and (2) using a public microarray data set and discussed its application for (3).

Program Nr: 2558 from 2003 ASHG Annual Meeting

**SNP haplotype analysis for qualitative and quantitative traits in unrelated individuals and parent-offspring trios.**

*S.M. Purcell*<sup>1,2</sup>, *M.J. Daly*<sup>1</sup>, *P.C. Sham*<sup>2</sup>. 1) Daly Lab, Whitehead Institute, Cambridge, MA; 2) Social, Genetic and Developmental Psychiatry Research Centre, Institute of Psychiatry, London, UK.

A flexible mixture-of-regressions framework is used to analyze SNP haplotype association data for qualitative (logistic regression) and quantitative (linear regression) traits. When parental genotype information is available, a between/within family partitioning of haplotypic effects is possible, which allows for a test of association robust to population stratification. Furthermore, a conditioning-on-trait-values approach allows the analysis of trait-ascertained samples, including affected-only parent-offspring designs. An additional, independent secondary test based on pair-wise haplotypic similarity can increase power. These methods, along with sliding window and permutation test approaches, are implemented in the WHAP software package. Advantages of this approach are evaluated in both real and simulated data.

**A Semiparametric Regression Model for Oligonucleotide Arrays.** *G. Yin*<sup>1</sup>, *J. Hu*<sup>2</sup>. 1) Biostatistics, M.D. Anderson Cancer Center, Houston, TX; 2) Biostatistics, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Oligonucleotide arrays can provide a broad measure of the expression levels of thousands of genes simultaneously. It is essential to develop methods to extract valuable information from the gene expression array data sets. The parametric model-based analysis of oligonucleotide expression arrays using the difference between the perfect match (PM) and mismatch (MM) probe intensities, namely (PM-MM), is very popular and widely used to calculate the model-based expression indexes (MBEI). However, the relationship between PM and MM may not be simply linear. The misspecified linearity may bring in bias and affect the accuracy of MBEI. Therefore, a semiparametric model incorporating the spline smoothing technique is proposed to study oligonucleotide gene expression data. No specific parametric functional form is assumed for mismatch probe intensities that allows much more flexibility in the fitted model. The model specification for oligonucleotide array data is particularly appealing since PM and MBEI are of major interest, while the effects of MM are nuisance. The new approach improves the model fitting, hence the estimation of MBEI. Extensive comparisons between the parametric (PM-MM) model and our proposal are made regarding different aspects including variances, model goodness-of-fit and assessment of the gene expression indexes. Control genes are very important and valuable in many facets of the comparisons. The proposed method is applied to a data set of 18 HuGeneFL arrays. It shows that the proposed semiparametric regression model is easy to implement and outperforms the popular parametric model with (PM-MM) as the response, in terms of capturing the gene expression indexes.

Program Nr: 2560 from 2003 ASHG Annual Meeting

**A novel non-parametric approach to the analysis of genetic haplotypes and genomic pathways.** *K.M. Wittkowski.*  
General Clinical Research Center, The Rockefeller University, New York, NY.

Few diseases can be explained by a single marker and few drug effects can be explained by looking at the expression of a single gene. In either case, the assumptions underlying the linear model are difficult to justify, so that results based on classical statistical methods are difficult to interpret and conclusions can easily be misleading. We present a new family of non-parametric statistical methods to overcome these challenges.

Most of the currently available statistical approaches are based on the linear model (Euclidian distance). Combining evidence from different markers or genes by means of linear combinations (counts of high risk alleles, average mRNA), however, requires that the variables (markers) are of (a) known relative importance, (b) constant correlation, and (c) specific functional form of interaction. Unfortunately, degree of linkage disequilibrium and type of interaction (from recessive over multiplicative to dominant) within a sequence of markers (haplotype) and between haplotypes are typically unknown, as are the correlations and interactions between genes responding to treatment.

The first approach allowing for the analysis of multivariate ordinal data to assess profiles of risk factors, safety indicators, and activity parameters was based on the marginal likelihood (JASA 1992, AJPH 1998) While eminently useful, it lacked computational efficiency. We now propose a new variant, where the computational effort raises only with the square of the number of subjects, rather than the factorial. The method is a member of a new class of non-parametric methods for genetic and genomic data, which is based on u-statistics (Hum Hered 2002).

We demonstrate applications of u-statistics for multivariate ordinal data in genetics and genomics using animal and clinical data from studies on atherosclerosis and psoriasis.

Program Nr: 2561 from 2003 ASHG Annual Meeting

**Genetic psychometrics: Concepts, methods, and examples.** *L.M. Evans, J.R. Kelsoe, N.J. Schork.* Dept Psychiatry, Univ California, San Diego, La Jolla, CA.

Psychometrics encompasses the development, validation, and interpretation of scales and questionnaire-like items meant to capture aspects of human behavior and personality. The statistical procedures used in the development and validation of psychometrical scales have a long and fruitful history. In using psychometrical scales in studies attempting to identify genetic factors that influence human behaviors and personalities, however, a number of issues arise. We outline a series of statistical techniques meant to facilitate and enhance the use of psychometrical scales in genetic studies. Each of the proposed techniques is motivated by the intuition that certain subsets of the subscales or items contributing to or defining a psychometrical scale may be more directly influenced by relevant genetic factors than others. Determining which subsets are more or less influenced by relevant genetic factors is therefore the goal of our proposed techniques. Although each of our proposed techniques deserves greater scrutiny, we consider four major areas of concentration: 1. determining homogenous subgroups of individuals based on a scale that may have common genetic backgrounds; 2. the identification of subscales and items which maximize heritability; 3. the identification of subscales and items which maximize linkage information; and 4. the identification of subscales and items which maximize genetic association. We showcase many of our proposed techniques with data derived from 29 bipolar disorder families.

**Finding haplotypes with NEMO.** *D.F. Gudbjartsson, A. Kong.* Decode Genetics, Reykjavik, Iceland.

A general method that uses NEsted MOdels for studying haplotype groups is introduced. This likelihood-based method is implemented in our program NEMO, which allows for many polymorphic markers, SNPs and microsatellites, and will be distributed for free upon publication. The method is designed for case-control and quantitative trait studies where the purpose is to identify haplotype groups that confer different risks. It is also a tool for studying LD structures.

When many markers are being investigated, apart from looking at each haplotype individually, meaningful summaries often require putting haplotypes into groups. A particular partition of the haplotype space is a model that assumes haplotypes within a group have the same risk, while haplotypes in different groups can have different risks. Two models/partitions are nested when one, the alternative, is a finer partition of the other, the null, i.e, the alternative model allows some haplotypes having the same risk in the null model to have different risks. Generalized likelihood ratio tests can be used to test the two models. One common way to handle uncertainty in phase and missing genotypes is a two-step process of first estimating haplotype counts and then treating the estimated counts as the exact counts, a method that can sometimes be problematic and may require randomization to properly compute p-values. In NEMO, maximum likelihood estimates, likelihood ratios and p-values are calculated directly for the observed data, treating it as a missing-data problem. NEMO allows complete flexibility for partitions. For example, suppose that allele 1 of two SNPs in LD is associated with the disease. The alternative grouping of [(0,0)], [(0,1)], [(1,0),(1,1)] versus the null grouping of [(0,0), (0,1)], [(1,0),(1,1)] can be used to test whether SNP2 is contributing to risk after adjusting for the effect of SNP1. Another meaningful test to perform is the alternative grouping of [(0,0)], [(0,1),(1,0)], [(1,1)] versus the null grouping of [(0,0)], [(0,1),(1,0),(1,1)]. Here the issue is whether the haplotype (1,1) has higher risk than (0,1) and (1,0). Analyzing our stroke data with NEMO reveals three groups of haplotypes; the wild type, a high-risk and a low-risk group.

**Visualization of large, complex human genealogy datasets: Drawing a pedigree from European royalty genealogy data.** *J.R. Garbe, Y. Da.* Animal Science, University of Minnesota, St. Paul, MN.

Human genetic analysis often starts with drawing a pedigree from genealogy data to show the population structure, the relationship among individuals, and gene flows from generation to generation. However, for large and complex populations, visualization of the genealogy data becomes a challenge. The purpose of this research is to study the feasibility of drawing pedigrees of large complex populations with Pedigraph, a pedigree drawing program developed by the authors ([www.animalgene.umn.edu](http://www.animalgene.umn.edu)). European royalty genealogy data from the Directory of Royal Genealogical Data ([www.dcs.hull.ac.uk/public/genealogy/royal/](http://www.dcs.hull.ac.uk/public/genealogy/royal/)) was used because it is a large, complex, publicly available dataset. The full pedigree of European royalty contains 48,605 individuals spread out over 106 generations, with entries as early as the first century AD and as recent as 1997. Pedigraph produced a drawing of the full pedigree on a 2 GHz computer in 80 minutes. Color lines connecting parents and offspring were used to enhance the visibility of parent-offspring relationships. The drawing gives a good sense of the overall structure of the full pedigree and reveals highly complex inbreeding relationships, a visual effect that is impossible from reading the genealogy data. Displaying the pedigree on a computer and using magnification allows a clear view of any part of the pedigree. However, to print out a legible copy would require a piece of paper 680 feet wide and 82 feet tall. By using Pedigraph's summarization option the width of the drawing can be reduced by two-thirds. Using Pedigraph's option of extracting partial pedigrees, the pedigree of Victoria Hanover, Queen of Britain, was drawn. This partial pedigree shows 3,975 ancestors and 1,148 descendants of the queen, and is viewable without the need of an excessively large printout. The results show that visualization of large complex genealogy datasets is possible, the main limitation being the size of the pedigree printout, which could be too large to be practical.

**A comparison between different strategies for computing confidence intervals for the linkage disequilibrium measure  $D'$ .** *S.K. Kim, K. Zhang, F. Sun.* Dept. of Biological Sciences, Univ. of Southern California, Los Angeles, CA.

Many linkage disequilibrium (LD) measures have been used to study LD patterns and for haplotype block partition. We examine the properties of one of these measures, Lewontins  $D'$ , in order to understand the dependency of its confidence interval to allele frequency and sample size as well as its applications in defining haplotype blocks. This measure and its confidence intervals were used to partition haplotypes into blocks by Gabriel et al. (2002) as well as in many other applications. Gabriel et al. (2002) utilized a bootstrap approach to calculate the confidence interval for  $D'$ . Under this method, over 1000 bootstrap samples maybe needed to obtain an accurate estimate of the confidence interval for each pair of single nucleotide polymorphism (SNP) markers which can be very computationally intensive, particularly when many SNP markers are involved. We develop two alternative methods for calculating the confidence interval for  $D'$  without bootstrap: one based on the approximate variance of  $D'$  given by Zapata et al. (1997) and the other based on a maximum likelihood estimate (MLE) of  $D'$  together with Fisher Information theory. Both methods depend on normal approximation for the estimates of  $D'$  for large sample sizes. We assess and compare the coverage of the confidence intervals using the three methods through extensive simulations. We define the coverage as the fraction of times the estimated confidence interval contains the value of  $D'$ . Under all simulated conditions, the average coverage of the bootstrap method is less than the pre-specified coverage. When the sample size is small (100), the remaining two methods slightly over estimate the coverage with Zapatas method having the smallest standard error among all three methods. When the sample size is large (200), the estimated coverage from both Zapatas and MLE methods are very close to the pre-specified coverage with the MLE method having the smallest standard error among all three methods. Thus, we recommend the use of Zapatas method when the sample size is small likewise the MLE method when the sample size is large for determining the confidence intervals.

Program Nr: 2565 from 2003 ASHG Annual Meeting

**Detection of gene-gene interactions in general pedigrees.** *J.H. Moore<sup>1</sup>, L.W. Hahn<sup>1</sup>, M. Bass<sup>2</sup>, E.R. Martin<sup>2</sup>.* 1) Program in Human Genetics, Vanderbilt University, Nashville, TN; 2) Center for Human Genetics, Duke University, Durham, NC.

The pedigree disequilibrium test (PDT) of Martin et al. (*AJHG* 67:146-54, 2000) has made it possible to carry out valid single-locus tests of linkage disequilibrium in extended pedigrees. The goal of this study is to modify the PDT method to allow the detection of multilocus effects and gene-gene interactions in extended pedigrees. To accomplish this goal, we have combined the PDT with the multifactor dimensionality reduction (MDR) approach of Ritchie et al. (*AJHG* 69:138-147, 2001). The MDR method was designed specifically for the detection of gene-gene interactions in case-control study designs using unrelated individuals. With MDR, the dependent variable is case-control status while the independent variables are multilocus genotypes. We have made the detection of gene-gene interactions in pedigrees possible by creating a new dependent variable based on within-family cases and controls. For family triads (affected offspring and both parents), transmitted and untransmitted multilocus genotypes are used. For families without parental data, multilocus genotypes of affected individuals are compared to their unaffected siblings. This new data encoding permits the MDR approach to identify the combination of genotypes that are positively associated with disease within families. As with the original MDR, cross-validation and permutation testing are used to estimate prediction errors, determine statistical significance, and control for type I error due to multiple comparisons. Using family triads simulated from nonlinear gene-gene interaction models, we have determined that the power of the new MDR-PDT approach (>80%) is equivalent to that of the original MDR approach when applied to case-control data generated from the same genetic models. Future studies will focus on simulations to evaluate the MDR-PDT in a variety of different pedigree structures. This new method provides a starting point for nonparametric and genetic model-free detection and characterization of nonlinear gene-gene interactions in general pedigrees.

Program Nr: 2566 from 2003 ASHG Annual Meeting

**PedStats: A utility for summarizing the contents of pedigree files with the ability to produce graphical output in PDF format.** *J.E. Wigginton, G.R. Abecasis.* Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI.

With the advent of single nucleotide polymorphisms (SNPs) and high throughput genotyping technologies, the size of the typical dataset used for pedigree analysis has increased dramatically - pedigree files with thousands of markers measured on hundreds of individuals are no longer unusual. With such large files, it is no longer practical to rely on manual inspection of data to spot patterns or anomalies. To address this issue, we've developed Pedstats, a C++ utility that produces text and graphical summaries of the family structure, trait and marker information contained in any pair of QTD, LINKAGE or MENDEL format pedigree and data files.

Pedstats checks input files for formatting and logical errors, generates summary statistics and plots of family size distributions, quantitative trait and covariate distributions, a listing of heterozygosity and allele frequency distributions for each marker, and breakdowns of affection status information. Additional features include an option to check Hardy-Weinberg equilibrium and produce a graphical synopsis of test results, the capability to calculate correlations and produce summary plots of trait distributions for different relative pairs, sex-specific output, and an option to remove uninformative individuals from pedigrees. All output can be written in either text or graphical PDF format; the latter has the virtue of being accessible and portable across a wide variety of computer platforms. Pedstats is distributed with the Merlin package, and is freely available for download at <http://www.sph.umich.edu/csg/abecasis>.

**Haplotype frequency and effect estimations for family-based association analyses.** *G. Xu<sup>1,2</sup>, Q. Yang<sup>1,3</sup>, S. Demissie<sup>3</sup>, R.H. Myers<sup>1,2</sup>.* 1) Neurology, Boston University Sch Medicine, Boston, MA; 2) Bioinformatics Program, Boston University, Boston, MA; 3) Department of Biostatistics, Boston University School of Public Health, Boston, MA.

In many cases of family-based haplotype association studies, the parental genotypes and phase for many individuals are missing. Here we present a method utilizing SNP data, to improve haplotype frequency estimation for extended pedigrees, and which may then be applied in a mixed regression model to estimate haplotype effects for quantitative traits. For founders with missing genotypes, haplotypes are inferred from offspring genotypes. Pairs of founder haplotypes, one for each chromosome, are retained when they are compatible with all offspring genotypes. Expectation maximization (EM) algorithm is used to estimate haplotype frequencies in the population using only the compatible founder haplotypes. Haplotype association is assessed as follows: the trait is the dependent variable, with the haplotypes as fixed effects and the pedigree as a random effect. Founders haplotype information is also included as a fixed factor to adjust for population admixture. A weighted regression approach is employed to accommodate the varying certainty of different haplotype configurations. Simulation studies are performed to compare the accuracy of haplotype frequency estimates using our method with those for existing approaches. Results from the mixed regression are compared with existing family-based association tests. We apply our method to obtain haplotype frequencies for SNPs in the PARK3 region, and the mixed regression to estimate the haplotype effects against onset-age of Parkinsons disease.

Program Nr: 2568 from 2003 ASHG Annual Meeting

**A Semiparametric Method for Estimating Population Size History from Sequence Data using Markov Chain Sampling.** *A. Renwick, M. Kimmel.* Dept Statistics, Rice Univ, Houston, TX.

We present a new, likelihood-based method of inferring population size history from DNA sequence data. The approach is, first, to devise an estimator that is a function of the (unobserved) genealogy of the sampled sequences, and, then, to compute the distribution of that estimator conditioned on the (observed) sequence data. This is accomplished by embedding the estimator in a Bayesian Markov Chain Monte Carlo method of phylogenetic inference. We estimate the parameter (four times the product of population size and mutation rate) as a piece-wise constant function of time. This allows the method to be "semiparametric" in the sense that it requires a parametric model of sequence evolution but does not impose any restrictions on the nature of the population size change history. This method takes advantage of the increasing availability of sequence data by accommodating an arbitrary number of unlinked loci. We illustrate the method with applications to anthropology and epidemiology.

**Linkage analysis allowing for errors - application to SNPs.** *D. Gordon*<sup>1</sup>, *C. Haynes*<sup>1</sup>, *C. He*<sup>2</sup>, *T. Matisse*<sup>2</sup>, *S. Finch*<sup>3</sup>, *J. Ott*<sup>1</sup>. 1) Lab of Statistical Genetics, Rockefeller University, New York, NY; 2) Department of Genetics, Rutgers University, Piscataway, NJ; 3) Department of Applied Math and Statistics, Stony Brook University, Stony Brook, NY.

Genotyping errors can significantly affect power and type I error rates when performing linkage analyses. Using a maximum likelihood approach, we have developed a method that performs linkage analyses in the presence of genotyping errors and which maintains a correct type I error rate, even in the presence of errors. In addition, our method allows for the estimation of locus specific error rates under the null hypothesis (recombination fraction  $\theta = 0.5$ ) and the alternative ( $\theta < 0.5$ ). Because our method offers a selection of multiple error models, model fitting using a generalized likelihood ratio statistic can be performed to determine what error model best fits the data when the marker locus is a SNP. We have developed a software program, Linkage-AE, that uses our method. Results of the model fitting procedure with a set of 1440 SNPs genotyped on over 500 individuals in several CEPH pedigrees using the error model proposed by Sobel Papp and Lange (SPL) indicate that the average probability of an error occurring in a SNP locus is  $5.5 \times 10^{-3}$ , with a median probability of  $5.5 \times 10^{-5}$ . The largest observed probability is 0.23. Also, we observe that the probability for an error in a SNP is greater than 0.01 for 7 percent of the 1440 SNPs typed. These results indicate that the distribution of probabilities is highly skewed. Finally, we find that the most general error model for SNPs consisting of 6 error parameters is a better fit than the SPL model for 20 out of 1440 markers. These results suggest that the SPL error model is good error model for most SNPs.

**Prospective and Retrospective Variance-Component Linkage Methods for Complex Trait Data.** *M.P. Epstein<sup>1</sup>, X. Lin<sup>2</sup>, M. Boehnke<sup>2</sup>*. 1) Dept of Human Genetics, Emory Univ, Atlanta, GA; 2) Dept of Biostatistics, Univ of Michigan, Ann Arbor, MI.

Variance-component (VC) linkage analysis is a powerful and flexible tool for the mapping of genes that influence quantitative traits. However, existing VC methods typically assume the trait data (1) either follow (or may be transformed to follow) a multivariate normal distribution within a family and (2) consist of a random sample from the population. Several papers have shown that violation of the normality assumption (1) may lead to biased parameter estimates and elevated type I error rates. Violation of the random-sampling assumption (2) also yields improper inference unless one adjusts the prospective likelihood of the VC method by the sampling criterion. Unfortunately, the complex sampling schemes typically applied in genetic studies may prevent accurate implementation of such a sampling adjustment in practice.

Given the appealing design of the VC method for random normal data, we have extended the method to accommodate both non-normal trait data and non-random sampling. For the first extension, we apply the generalized-linear-mixed-modeling (GLMM) framework to accommodate trait data with known non-normal distributions, such as binary (e.g. disease) and count data. For the latter extension, we develop a retrospective variance-component framework that is independent of the sampling criterion. We believe these two extensions provide a unified VC framework that allows prospective and retrospective analyses of genetic trait data that follow a variety of known distributions. We illustrate the utility of these extensions using simulated data and quantitative trait data from the Finland-United States Investigation of NIDDM (FUSION) study.

Program Nr: 2571 from 2003 ASHG Annual Meeting

**Unification of variance components and Haseman-Elston regression for quantitative trait linkage analysis in humans.** *W.M. Chen, K.W. Broman, K.Y. Liang.* Department of Biostatistics, Johns Hopkins Univ, Baltimore, MD.

There are two major approaches for linkage analysis with quantitative traits in humans: variance components and Haseman-Elston regression. Previously, these have been viewed as quite separate methods. We describe a general model for quantitative trait linkage analysis in general pedigrees, fit by use of generalized estimating equations (GEE), for which the variance components and Haseman-Elston methods (including the many extensions to the original Haseman-Elston method) are special cases, corresponding to different choices for the working covariance matrix.

These results have several important implications. First, this work provides new insight regarding the connection between these methods. Second, asymptotic approximations for power and sample size allow clear comparisons regarding the relative efficiency of the different methods. Third, our general framework suggests important extensions to the Haseman-Elston approach which make more complete use of the data in extended pedigrees and a natural incorporation of environmental and other covariates, while maintaining the valuable robustness properties of Haseman-Elston regression.

**How multiple data sets are combined affects autism genome screen results: 'Pooled' compared to 'sequentially updated' linkage analyses.** *R. Goedken<sup>1</sup>, J. Piven<sup>2</sup>, V. Sheffield<sup>1</sup>, T. Wassink<sup>1</sup>, J. Beck<sup>1</sup>, V. Vieland<sup>1</sup>*. 1) University of Iowa, Iowa City, IA; 2) University of North Carolina, Chapel Hill, NC.

Under conditions of intra- and inter-sample heterogeneity, pooling (concatenating) data sets and computing overall summary linkage statistics will tend to wash out true linkage signals (Vieland et al., 2001). But for standard model-free and model-based linkage statistics, pooling data sets prior to analysis is the only way to measure the aggregate strength of evidence taking all available families into consideration at once. By contrast, the posterior probability of linkage (PPL) is a mathematically rigorous approach to accumulating evidence across heterogeneous data sets via sequential updating. We illustrate the effects of data-pooling by reanalyzing genome screen data for 119 (primarily) nuclear families ascertained on the basis of at least two autistic children. The data were separated a priori into 4 subsets based on temporal and clinical factors. Complete PPL results are presented elsewhere (Vieland et al., this meeting). Here we compare the 2-point PPL results (updated) with results from pooling all families (pooled) and using either model-free multipoint analyses (ASPEX MLSs), multipoint HLODs maximized over one dominant, one recessive model (M-MMLS), or 2-point MMLS HLODs (MMLS). A very different picture is obtained across the genome depending on the method used to handle the 4 data sets. For example, on chromosome 16 we obtain a PPL of 70% in the full set of 119 families, with virtually all of the linkage signal coming from subset 1 (PPL = 77%). In subset 1 at this location we get (on the LOD scale): MLS 1.6, M-MMLS 3.3, MMLS 3.8. Pooling all 119 families we get: MLS 0.5, M-MMLS 0.7, MMLS 1.4. This represents 63%-79% drop in the lod score relative to subset 1 alone, compared to only 10% drop in the PPL. We do not know whether this is a true linkage, but it is clear that failure to appropriately accumulate evidence across disparate data sets can alter the overall conclusions of a genome screen.

Program Nr: 2573 from 2003 ASHG Annual Meeting

**A Model-Based Bayesian Transmission Disequilibrium Approach For Testing Linkage.** *V. George*<sup>1</sup>, *S. Shete*<sup>2</sup>. 1) Biostatistics, Ryals PHB #327, Univ Alabama at Birmingham, Birmingham, AL; 2) Epidemiology, Box 189, UT M. D. Anderson Cancer Center, Houston, TX.

The transmission disequilibrium test (TDT) is a powerful method for detecting a disease locus in linkage and linkage disequilibrium with a marker. Here, assuming the knowledge of mode of inheritance, we developed a Bayesian method for testing for linkage in the presence of allelic association. We used Markov chain Monte Carlo simulation approach to fit the proposed Bayesian model. We compared the proposed Bayesian TDT with the usual TDT and found that the proposed method is more powerful and that the estimated values of recombination fraction are more accurate compared to existing method. We evaluated robustness of our method when mode of inheritance is not correctly specified.

**Linkage Analysis with Adjustment for Associated Marker Genotypes in Fine-Mapping Studies.** *R.L. Hanson, W.C. Knowler.* DAES, NIDDK, Phoenix, AZ.

Association studies are often used to finely map loci identified by linkage analysis. Once a marker associated with the trait is identified, linkage analysis with adjustment for marker genotypes may help determine if the association explains the linkage. We conducted a simulation study to determine sensitivity of this procedure to extent of linkage disequilibrium and to similarity in frequency between functional and marker alleles. Families consisted of 264 nuclear families, containing 1004 offspring and 1893 sib-pairs who had participated in a linkage study. Genotypes were simulated for these individuals at: 1) a diallelic functional locus with a high-risk allele with frequency= $f_A$ ; 2) a diallelic marker locus closely linked ( $=0.00$ ) to the first with an allele of frequency= $f_1$  associated with the high-risk allele at a specified degree of linkage disequilibrium ( $D'$ ); 3) a perfectly informative marker linked ( $=0.05$ ) to the other two loci. Trait values were generated for a quantitative trait under the assumption that heritability specific to the functional locus was 25%. 500 replicates were generated for each value of  $f_A$ ,  $f_1$  and  $D'$ . The regression method of DeFries and Fulker was used to evaluate linkage between the trait and the perfectly informative marker. The LOD score from a model that included covariates for the diallelic marker was compared with the unadjusted LOD. When diallelic marker and functional loci were identical ( $D'=1.0$ ,  $f_A=f_1$ ), adjustment for the marker completely attenuated evidence for linkage, but the extent of attenuation diminished when  $D'<1.0$  and  $f_A f_1$ . For example, when  $f_A=f_1=0.2$ , median adjusted LOD=2.1 when  $D'=0.0$  (equivalent to the unadjusted LOD), while the adjusted LOD=1.4 when  $D'=0.4$ , LOD=0.6 when  $D'=0.8$  and LOD=0.0 when  $D'=1.0$ . When  $f_A=0.2$  and  $D'=0.8$ , median adjusted LOD=1.4 when  $f_1=0.4$ , LOD=1.7 when  $f_1=0.6$  and LOD=1.9 when  $f_1=0.8$ . Linkage analysis with adjustment for marker genotypes can determine if a marker accounts for the observed linkage and, thus, may be the variant directly influencing the trait. However, conditioning linkage on association with a marker in the disease gene but not identical to the causal variant and having a different allele frequency will not completely account for the linkage.

**Comparative Study of multipoint methods for genotype error detection.** *N. Mukhopadhyay<sup>1</sup>, S.G. Buxbaum<sup>1</sup>, D.E. Weeks<sup>1,2</sup>.* 1) Dept Human Genetics, Univ Pittsburgh/Sch Pub Health, Pittsburgh, PA; 2) Dept Biostatistics, Univ Pittsburgh/Sch Pub Health, Pittsburgh, PA.

Several programs, such as Merlin, SibMed, SimWalk2 and Mendel4, provide multipoint estimation of the posterior probability of genotyping errors. Very little has been done to characterize their relative behavior, power, and effects of marker spacing, pedigree structure, and size. Our simulation study is designed to address these issues.

Input data consists of 4 nuclear pedigree structures, with and without typed parents, and one with a half-sibling. Marker data consists of 11 microsatellite markers with realistic allele frequencies, and map spacings of 1, 2, and 5 cM. We simulated 5,000 replicates of each pedigree structure and marker map. About 4% of the middle marker's genotypes were switched to another genotype with uniform probability.

Initial results are from Merlin's mistyping option. Merlin assigns a low likelihood score to genotypes to indicate mistyping. We use the lower 5th percentile of Merlin scores for pedigrees without input errors as a threshold for flagging mistypings. While Merlin's default threshold is 0.025, our values range from 0.53-0.75, varying slightly by pedigree structure and marker spacing. Power to detect Mendelianly consistent errors ranges from 44%-68% decreasing with marker density. Increasing map density from 2 cM to 1 cM does not affect cutoffs or power. Pinpointing the mistyped person in a pedigree is difficult; often, a relative without erroneous genotypes is also implicated. Mistyping at a locus causes flanking markers to have an elevated false positive rate.

We are performing similar comparative studies of other error detection programs. The challenge lies in reconciling the different characterizations of genotype errors, e.g., Merlin gives likelihoods for each genotype, whereas SibMed gives the posterior genotyping error probability for a sib-pair. Our initial study is aimed at characterizing a common set of performance parameters so as to enable such comparisons.

**Comparison of Multiple SNPs and Single Microsatellites for GenomeScans.** *E. Lindholm*<sup>1</sup>, *D.A. Greenberg*<sup>1,2</sup>, *S.E. Hodge*<sup>1,2</sup>. 1) Div Stat Genetics, Columbia U; 2) NYSPI, NY, NY.

Single nucleotide polymorphisms (SNPs) are biallelic and therefore not as polymorphic as microsatellites. However, by analyzing several closely-spaced SNPs, we can increase the information content for linkage. We investigated how the lod score depends on the number of SNPs used in a linkage analysis and compared the results to information from a single polymorphic microsatellite. We examined sib pairs (without parental information) and nuclear families with varying numbers of children. We simulated 20 data sets of 50 families each with 2-5 children per family. Marker data came from 1-10 SNPs with no recombination between them and in linkage equilibrium. We generated comparable data for one microsatellite with between 3 and 9 equally frequent alleles. Allele frequencies for SNPs were either 0.5 and 0.25 and were the same for all SNP loci in an analysis. GENEHUNTER was used to calculate lod scores (see table). We found that 4 or 5 closely spaced SNPs (depending on the allele frequency) were approximately equivalent to one microsatellite with 9 equally frequent alleles. Adding more than 5 or 6 SNP loci did not improve the lod score. More than 5 SNP markers will not increase the amount of linkage information but increasing the polymorphicity of a microsatellite beyond the 9 alleles we tested will increase the information. The results indicate that about 1200-1500 SNPs would be enough to get the same information as 300 microsatellite markers for linkage analysis in nuclear families.

#SNP	2ch.	3ch.	5ch.	SIBS		#All	2ch.	3ch.	5ch.	SIBS
2	9.0	18.8	37.7	2.9		4	10.2	20.2	40.0	3.4
5	14.3	28.7	57.8	4.8		6	12.1	23.9	47.5	4.4
10	15.1	30.0	60.1	6.0		9	13.0	26.5	51.9	5.1

**A likelihood ratio test for linkage with covariates based on IBD allele sharing in affected relatives.** *L. Mirea*<sup>1</sup>, *L. Briollais*<sup>1,2</sup>, *S.B. Bull*<sup>1,2</sup>. 1) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; 2) Department of Public Health Sciences, University of Toronto, Toronto, ON, Canada.

Gene-mapping studies of complex traits often employ linkage methods that do not model possible differences in *identical-by-descent* (IBD) allele sharing of affected relatives among sampled families. Statistical power of linkage tests can be increased by including covariates associated with different genetic mechanisms that cause variation in allele sharing. We investigate a particular extension of the one-parameter exponential likelihood model defined by Kong and Cox (1997 Allele-sharing models: LOD scores and accurate linkage tests. *Am J Hum Genet* 61:1179-1188) that, in addition to the linkage parameter ( $\theta$ ), incorporates a parameter ( $\delta$ ) measuring deviation in IBD allele-sharing between two groups of families classified by a binary covariate. Based on this extension, a likelihood ratio statistic can be formulated to test departures from the null of no linkage ( $\theta=0$ ) and no differences in allele sharing ( $\delta=0$ ) between the two covariate-defined family groups. Under asymptotic regularity conditions, the significance of the two-parameter test for linkage with the covariate ( $\theta$  or  $\delta$ ) can be evaluated using a  $\chi^2$  distribution with 2 df. In comparison, the test for linkage in the one-parameter exponential model (Kong and Cox, 1997) evaluates the square root of the likelihood ratio statistic with respect to a Gaussian distribution and considers a one-sided alternative ( $\theta > 0$ ). The linkage tests with and without the covariate were applied to family data simulated under a model of locus heterogeneity such that each replication included a mixture of linked and unlinked families with affected siblings and cousin pairs. In scenarios where the family-level covariate correctly identified linked and unlinked families, the test with the covariate can have greater power than the test without the covariate, particularly when the relative number of linked families is small and differences in allele sharing are large. Conversely, when the covariate is independent of group differences in allele sharing, power is reduced for the test with the covariate compared to the test without.

Program Nr: 2578 from 2003 ASHG Annual Meeting

**Ascertainment Corrections in Variance Component Analysis.** *J. Peng, D. Siegmund.* Statistics Department, Stanford University, Stanford, CA 94305.

Variance component methods to map quantitative trait loci require the estimation of various nuisance parameters, e.g., the trait mean, variance, and correlations between relatives. This is easily accomplished when pedigrees are randomly ascertained. Here we study ascertainment adjustments for pedigrees consisting of  $s$  siblings (with or without parents), which are ascertained on the basis of phenotypic values of  $r < s$  of the siblings. We show that two different schemes of ascertainment correction, (i) conditioning on trait values and (ii) conditioning on the event of ascertainment, are asymptotically equivalent when the number of sibships is large. We also find that of the three important nuisance parameters, the estimate of the phenotypic variance is robust against failure to correct for ascertainment, but the estimates of the phenotypic correlation and mean are not robust. Failure to adjust adequately estimates of the phenotypic mean and correlation can lead to a substantial loss of power, while failure to adjust estimates of the variance leads to negligible loss of power.

**Information Content of Multi-point Analysis and Efficiency of Two Stage Genotyping.** *D. Siegmund<sup>1</sup>, J. Peng<sup>1</sup>, H.K. Tang<sup>2</sup>*. 1) Dept Statistics, Stanford Univ, Stanford, CA; 2) Hewlett Packard Corporation, Palo Alto, CA.

By extensive simulation we examine the efficacy of multipoint methods to compensate for markers that are incompletely polymorphic (a) when pedigree founders are available for genotyping and determination of identity-by-descent (IBD) of affecteds is conditional on founder genotypes and (b) when founders are unavailable and IBD status is determined through allele frequencies. When markers are reasonably informative and closely spaced and allele frequencies are correctly specified, relatively little information is lost when founders are not genotyped; but when markers are only moderately informative, knowledge of founder genotypes can add substantially to the available IBD information. We also consider genotyping in two stages: in the first stage an intermarker distance of 20 cM is used, and in the second stage the distance is reduced to 5 cM in the neighborhood of any marker yielding a Z-score above  $z_0$  in the first stage. For the case of a single disease gene that can be detected by a 5 cM genome scan with power 0.67 or 0.9, and for  $z_0 = 1.28$  or 1.65, we find that the gene can be detected with no loss of power and 1/3 as many genotypes, except when founders are not genotyped and marker polymorphism is only moderate. In order to obtain accurate estimates of the genome wide significance level, we have used an importance sampling technique that may prove useful more generally.

**Effects of ascertainment on the power of variations of the Haseman-Elston method for a quantitative trait.** Y. Yao<sup>1</sup>, A.J.M. Sorant<sup>2</sup>, A.F. Wilson<sup>2</sup>. 1) Dept Epidemiology, Johns Hopkins Univ, Baltimore, MD; 2) IDRIB, NHGRI, NIH, Baltimore, MD.

Previous work has shown that non-random ascertainment of family data may affect the power of some methods of linkage analysis. The present study examined the effect of non-random ascertainment on the power of several versions of the Haseman-Elston (HE) method of model-independent sib pair linkage analysis, which all perform a regression on the estimated proportion of alleles shared identically by descent, using a different dependent variable for each version. The traditional HE method uses  $-.5$  times the square of the trait difference between sibs as the dependent variable (DIFF). Similarly, the revised HE method uses the mean-corrected cross-product (PROD). Other versions use half the square of the mean-corrected sum (SUM) or a weighted combination of the squared trait difference and the squared mean-corrected sum (W2) as the dependent variable. Model-independent linkage analysis was performed using each dependent variable in simulated samples of 100 nuclear families of sibships of size 4 with a quantitative trait generated under several genetic models: additive genetic heritability of 10% - 50%, with and without an additional polygenic or common sibship component. Four different ascertainment schemes were considered: random sampling (RS); selecting families with at least one sib with a high trait value (H1); at least two sibs with high values (H2); and families with extremely discordant sibs (ED). Results were summarized by counting p-values less than a critical value of .01 over 2000 replications of each experiment. For all HE analysis methods and all genetic models considered, the ED ascertainment scheme had the greatest power (169% better than RS on the average). The H1 and H2 schemes often improved power over RS for the PROD, DIFF and W2 methods. In all situations considered, the SUM method consistently had the worst power. PROD, DIFF, and W2 analysis version provided better power, with each of these methods having higher power than the others under different circumstances.

**Assessment and application of a nonparametric confidence set approach based on the mean test.** *C. Papachristou, S. Lin.* Department of Statistics, Ohio State University, Columbus, OH.

In genomewide linkage analyses one needs to adjust for the number of simultaneous hypotheses tested. Such an adjustment is very difficult due to the complex nature of dependencies amongst data arising from linkage studies. It is possible, though, to avoid dealing with multiple testing issues, by constructing confidence sets of markers tightly linked to a genetic trait through testing hypotheses that are reversal of the usual setup. Here, we examine the effect of several factors on the confidence set derived using the nonparametric mean statistic based on IBD sharing of affected sib pairs. Simulations are performed to assess the performance of the approach in terms of its true and false discovery rates, under varying conditions of the underlying single locus disease model, the amount of data, the heterozygosity of the marker, and the number of family members genotyped at the marker locus. We also provide rough guidelines for the choice of coverage probability of the confidence set that would lead to a false discovery rate of a desirable level. Our methods are then applied to the simulated data from the Genetic Analysis Workshop (GAW) 13, focusing on the high blood pressure trait. Our simulation results show that when the assumption of a single locus trait with accurate risk estimates is met, the confidence set is able to sufficiently localize the disease causing gene. However, a moderate increase of about 30% in the amount of data is necessary for the method to achieve the same power as when the marker is 100% polymorphic. Also, inaccurate estimation of the risks may compromise the power or inflate the false discovery rate of the method, particularly when genetics only plays a limited role in the disease causing mechanism. Incorrect specification of the number of disease causing genes also reduces the power of the method. Nevertheless, the results for the GAW data suggest that, despite potential reduction in power due to deviation from the ideal situation, our method can still achieve significantly higher power than standard nonparametric methods while maintaining comparable false positive rates, especially when the sample size is reasonably large.

**Detecting Gene-Gene Interactions: Linkage Designs versus Association Designs.** *S. Wang*<sup>1</sup>, *H. Zhao*<sup>1,2</sup>. 1) Department of Epidemiology & Public Health, Yale Univ, New Haven, CT; 2) Department of Genetics, Yale Univ, New Haven, CT.

Gene-gene interaction has received much attention recently both due to technological advances that have enabled researchers to study many genes simultaneously and due to a deeper appreciation of the complexity underlying human diseases with most human traits depending on several interacting genes as well as environmental factors. Gauderman (2002) and Wang and Zhao (2003, in press) have reported systematic studies on the statistical power to detect gene-gene interaction in association studies. In this presentation, we conduct a systematic study to evaluate the statistical power to uncover gene-gene interactions in linkage studies, with a focus on the affected sib pair (ASP) approach. We assume a logistic model for disease risk, and detect gene-gene interactions through correlation based on alleles shared identical-by-decent (IBD) at the two disease loci. In addition to logistic models, we also investigate the power to detect gene-gene interactions of several two-locus models with fixed penetrance effects that cannot be parameterized in logistic forms. The results of the ASP design are compared with the results of the association designs reported in Wang and Zhao (2003). Our results indicate that the association designs are far more powerful than the ASP designs for all the genetic models considered.

**Empirical Bayes Method for Incorporating Data from Multiple Genome Scans.** *T.M. Beasley, H. Wiener, D.B. Allison.* Biostatistics, University of Alabama, Birmingham, AL.

Individual genome scans can produce markedly biased estimates of QTL effects. Further, the confidence interval for their location is often prohibitively large for subsequent fine mapping and positional cloning. Given that a large number of genome scans have been conducted, not to mention the large number of variables and subsets tested, it is difficult to confidently rule out Type I error as an explanation for significant effects even when there is apparent replication in a separate data set. We adapted Empirical Bayes (EB) methods (Morris 1983) to analyze multiple datasets simultaneously and alleviate these problems while allowing for different QTL population effects across studies. We evaluated this method applied to meta-analyses of linkage studies via simulation, using actual marker spacing and allele frequencies on chromosome 11 from the NIMH Alzheimers Diseases Genetics Initiative. Under complete null conditions, the one-sided 95% rejection cutoff was close to the expected 1.64, when only one study was analyzed. For the EB method, the null cutoff value depended on the number of studies used to update the study of interest. For 5, 9, and 19 background studies respectively, the 95% cutoff values were 1.38, 1.22, and 1.05, respectively. These values were used to determine statistical significant for the non-null models. For non-null situations, phenotypes were assigned assuming 5% heritability. For data from one study, approximately 53% of the results were significant at a marker located at the same position as the affective locus. When the EB method was applied, these rejection rates rose to 78%, 91%, and 98% for 5, 9, and 19 background studies, respectively. At a marker 90cM from the affective locus, the rejection rates fell between 6.1% and 7.2%. When we compared single study results, EB results with 5 background studies, and a single study with six times the number of sibling pairs as the original study, the rejection rates for the EB method were always between the values for the other two situations. This EB method for incorporating data from multiple genome scans seems promising in that it has more power and better Type I error control.

Program Nr: 2584 from 2003 ASHG Annual Meeting

**Genomic Imprinting and Linkage Test for Quantitative Trait Loci in Extended Pedigrees.** *S. Shete, X. Zhou, C. Amos.* Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX.

Genomic imprinting is a mechanism in which only one of the two copies of a gene is expressed. Some genes that affect development and behavior in mammals are known to be imprinted. Deregulation of imprinted genes has been found in a number of human diseases. Incorporating imprinting information into linkage analysis results in a more powerful test for linkage. Here we propose an efficient method to test for linkage and imprinting of quantitative traits in extended pedigrees. We compared the results obtained by using the extended pedigree analysis approach proposed here to other existing approaches. We found that the proposed method is more powerful and uses extended pedigree information most efficiently.

**Resampling-based statistical methods to improve the estimation of locus-specific effects from genome-wide studies.** *L. Sun*<sup>1,2</sup>, *S.B. Bull*<sup>1,3</sup>. 1) Dept Public Health Sci, University of Toronto, Toronto, ON, Canada; 2) Programs in Genetics and Genomics, Hospital for Sick Children, Toronto, Ontario, Canada; 3) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada.

The accuracy of locus-specific effect estimates has emerged as one of the major methodological concerns in studies aimed at dissecting the genetic components of complex diseases and quantitative traits using genome-wide approaches. Some authors (e.g. Goring et al. 2001) have argued that reliable locus-specific parameter estimates can only be obtained in an independent sample; others (e.g. Allison et al. 2002) have shown that statistical methods such as moment-based procedure may be applied to the initial sample while reducing the estimation bias in some cases. Statistical resampling techniques such as cross-validation and the bootstrap have been successfully employed to address over-fitting and variable selection bias in diagnostic and prognostic prediction models in clinical settings and in microarray data analysis of gene expression. We hypothesize that, in the context of genome-wide genetic studies, these statistical techniques will also improve the estimation of locus-specific effects. In addition, they will be effective in the validation and interpretation of the initial findings. We examine different resampling techniques, such as 50-50 and 90-10 split-sample, 10-fold and 10x10-fold cross-validation, leave-one-out jackknife and bootstrap methods, and compare them with the naive method in which all data are used for both hypothesis testing and parameter estimation. Under a simple model, we derive analytically the upward bias of the naive estimator, and we show numerically the effectiveness of incorporating resampling techniques into estimation. We also consider a more realistic example in the context of genome-wide linkage analysis. Results of our preliminary simulation studies suggest that cross-validation and bootstrap can effectively reduce the estimation bias while retaining comparable power to detect linkage.

**The number of STR markers required to resolve relationships between pairs of individuals.** *S. Presciuttini*<sup>1,2</sup>, *C. Toni*<sup>3</sup>, *I. Spinetti*<sup>3</sup>, *R. Domenici*<sup>3</sup>, *J.E. Bailey-Wilson*<sup>2</sup>. 1) Dept Biomedicine, Univ Pisa, Italy; 2) Inherited Disease Research Branch, NHGRI, NIH, Baltimore MD, USA; 3) Unit of Legal Medicine, University of Pisa, Italy.

The inference about the biological relationship between pairs of individuals using genetic markers plays a central role in many areas of human genetics. We investigated the number of markers (*M*) necessary to assign a given proportion of pairs ( $1 - \alpha = 90\%$ ,  $95\%$ , and  $99\%$ ) to their correct relationship at three significance levels ( $\alpha = 0.01$ ,  $0.001$ , and  $0.0001$ ) against several alternative hypotheses. The following relationships were considered: 1) parent-child; 2) full sibs; 3) second degree, including half-sibs, grandparent-grandchild and avuncular pairs; 4) first cousins; and 5) non-relatives. For each relationship, 10,000 true pairs were simulated, and the likelihood ratio that each pair was falsely attributed to the other relationships was calculated using exact Bayesian probability equations. Simulations were carried out separately for 25 unlinked markers commonly used in the forensic practice, which were repeated a second time to reach a total number of 50 markers.

Full sibs are discriminated from non-relatives and first cousins with a relatively small number of markers even in the strictest conditions ( $1 - \alpha = 99\%$ ,  $\alpha = 0.0001$ :  $M = 32$  and  $24$ , respectively), whereas discrimination between full sibs and second-degree relatives (e.g., half sibs) is possible at  $\alpha = 0.001$  (with  $M = 42$ ); in contrast, discriminating between second-degree and more distant relationships is not practicable with only 50 independent markers. We also investigated the reduction in the number of total typed genotypes achieved by using a sequential test, based on a first set of ten markers and the addition of other sets of five markers each, while keeping  $1 - \alpha$  constant. Our analysis may be useful to assess the feasibility of studies in which genotyping is specifically performed for the purpose of verifying relationships, such as follow-up studies in linkage analysis (when genome-wide scan data are not available) and field studies in genetic anthropology.

**Estimation of critical values for genome scans.** *S. Bacanu*. Dept Psychiatry, WPIC, University of Pittsburgh Medical Ctr, Pittsburgh, PA.

It is widely recognized that the Lander-Kruglyak critical values for significant and suggestive linkage are very conservative for many linkage studies. Their conservativeness is due to imperfect information about recombination caused by limited polymorphism of markers and small, incomplete families. Simulation-based estimation of critical values is one route to obtain more pertinent thresholds. Simulation methods have the disadvantage that they are time consuming and require skill to integrate many different software/tools and results. I have been examining a different method to determine study-specific critical values. This approach uses the realized genome scan statistic data (standard normal NPL-scores) to estimate the suggestive and critical values. Stationary autoregressive models of increasing order  $p$  (AR( $p$ )) are fit to the standard normal statistics contained in the genome scan; the optimal AR order is determined via Bayesian Information Criterion (BIC). The estimated parameters of the optimal AR( $p$ ) are used to determine the estimated correlation between statistics for two adjacent markers. This correlation is used to determine the probability of up-crossing a certain boundary between any two markers on the map and, consequently, to estimate the critical values. The behavior of the proposed method is assessed via simulations of data sets based on marker allele frequencies and maps derived from Marshfield Screen Set 9 (MSS9). Three marker densities are considered in the simulations with average distance between markers of 5, 10 and 20cM. The 5 cM map is obtained by inserting a marker between any two MSS9 markers and the 20cM map is obtained by deleting every other marker from the (roughly) 10cM MSS9 map. Preliminary studies show promising results for the significant critical value but not for the suggestive one when linkage studies results are fitted by using an AR(1) process. Higher order AR (and, possibly, ARIMA) approximations are currently being investigated and the results will be presented. If this approach proves successful, it can easily be encoded in software for rapid determination of study-specific critical values.

**High Resolution Association Studies of Complex Diseases Using Parents as Controls.** *R. Fan<sup>1</sup>, M. Knapp<sup>2</sup>, C. Zhao<sup>1</sup>, M. Xiong<sup>3</sup>.* 1) Dept of Statistics, Texas A&M Univ, College Station, TX; 2) Institute of Medical Biometry, Informatics and Epidemiology, University of Bonn, D-53105 Bonn; 3) Human Genetics Center, University of Texas-Houston, TX.

Case control studies using unrelated controls are prone to false positive due to inappropriate controls, which can occur if there are population admixture or stratification. Alternatively, parental controls can be a valid option (Falk and Rubinstein 1987; Ott 1989; Schaid and Rowland 1998; Spielman, McGinnis, Ewens 1993). For parental controls, the methods of two-sample Hotelling's  $T^2$  statistic tests proposed by Fan and Knapp (2003) and Xiong, Zhao and Boerwinkle (2002) are not valid since cases and controls are correlated to each other. Not only the correlation between the haplotype blocks or markers needs to be taken into account, but also the correlation within each parent-offspring pair needs to be dealt with. One method is to perform paired Hotelling's  $T^2$  statistic tests, instead of the two-sample Hotelling's  $T^2$  statistic tests. The paired Hotelling's  $T^2$  test statistics are proposed for high resolution association study based on two coding methods: haplotype/allele coding and genotype coding, if data of multiple bi-allelic or multi-allelic markers or haplotype blocks are available. The validity of the proposed method is justified by rigorous mathematical and statistical proof under the large sample theory. The non-centrality parameters of the test statistics are calculated for power and sample size comparisons.

**Simultaneous estimation of haplotype frequencies, diplotype configurations, and diplotype-based penetrances for testing association between phenotypes and the presence of haplotypes using genotype and phenotype data. T.**

*Ito*<sup>1,2</sup>, *E. Inoue*<sup>2</sup>, *N. Kamatani*<sup>3</sup>. 1) Safety Science and Policy Dep., Mitsubishi Research Institute, Tokyo, Japan; 2) Algorithm Team, Japan Biological Information Research Center (JBIRC), Japan Biological Informatics Consortium (JBIC), Tokyo, Japan; 3) Division of Genomic Medicine, Department of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan.

Analysis of the association between haplotypes and phenotypes is becoming increasingly important. We have created an EM (expectation-maximization)-based algorithm to test the association between a phenotype and the presence of haplotypes, and to estimate diplotype-based penetrance using individual genotype and phenotype data from cohort studies and clinical trials. The algorithm estimates, in addition to haplotype frequencies, penetrances for subjects with a given haplotype and those without it. Relative risk can thus also be estimated. The maximum likelihood under the assumption of no association between the phenotype and presence of the haplotype ( $L_{0\max}$ ) (null hypothesis; i.e. with only 1 penetrance) and the maximum likelihood under the assumption of association ( $L_{\max}$ ) (alternative hypothesis; i.e. with 2 penetrances) were calculated. The statistic  $-2\log(L_{0\max}/L_{\max})$  was used to test the association. The present algorithm was implemented in the computer program PENHAPLO. Results of analysis of simulated data indicated that the statistic followed the  $\chi^2$  distribution with 1 degree of freedom asymptotically when the data were produced under the null hypothesis. The test had considerable power under certain conditions. Analyses of 2 real data sets from cohort studies, one concerning the *MTHFR* gene and the other the *NAT2* gene, revealed significant associations between the presence of haplotypes and occurrence of side effects. We propose a method to calculate the probability that a subject with genotype data but without phenotype data develops the phenotype based on estimated penetrances. Our algorithm may be especially useful for analyzing data concerning the association between genetic information and individual responses to drugs.

**Shannon's mutual information applied to population-based gene mapping.** *J.C. Mueller<sup>1</sup>, E. Bresch<sup>2</sup>, Z. Dawy<sup>2</sup>, T. Bettecken<sup>1</sup>, T. Meitinger<sup>1</sup>, J. Hagenauer<sup>2</sup>.* 1) Institute of Human Genetics, GSF, Munich, Germany; 2) Institute for Communication Engineering, University of Technology, Munich, Germany.

Correspondence: jakob.mueller@gsf.de

Connecting phenotype with genotype is a fundamental aim of genetics. In complex traits however there are many yet unknown factors influencing the genotype-phenotype relationship. Even without the environmental effect, the behavior of genetic association patterns is expected to be complex with multiple contributing loci, epistatic effects among loci, regulation feedbacks, and different genetic transmission systems. It is therefore important to develop parameter- and assumption- free methods for genotype-phenotype association studies. Shannon's mutual information, which determines the correlation between random variables, is such a simple and direct way of measuring the information relationship among genotypes and phenotypes in complex and unknown settings. In this paper we propose and describe the basic method for case-control studies. Simulated data sets with known causal loci are used to test its characteristics and power. The method performs well with different underlying transmission modes of the causal locus and is comparable to other state-of-the-art association tests. But the advantage of our method lies in its generality, its lack of assumptions and its capability of being extended to more complex settings like multiple phenotypes, multi-locus markers, and the joint investigation of interdependent markers.

Program Nr: 2591 from 2003 ASHG Annual Meeting

**Finding the etiological combination of SNPs in a candidate gene.** *A. Jannot, E. Genin, F. Clerget-Darpoux.* Unite 535, INSERM, Villejuif, France.

Numerous studies have underlined the complexity of the relationships between gene polymorphisms and diseases and the necessity to develop appropriate methods. The SNP combination test was developed to detect the role of a gene in the presence of SNP interactions within the gene. It tests the association between a quantitative trait and all possible phased combinations of various number of SNPs taking into account tests dependency. We extend the principle of this strategy to qualitative data. We study through simulations the power of this extension to detect the role of a gene when several SNPs are involved and interact. We also evaluate the capacity of this test to discriminate between the functional combination of SNPs and SNPs that are in linkage disequilibrium. The simulations performed are based on genotypic data of a study showing the role of several interacting SNPs on P-selectin gene in coronary diseases. We demonstrate that the SNP combination test is useful both for detection and fine modeling of genetic risk factors, and particularly in complex situations.

Program Nr: 2592 from 2003 ASHG Annual Meeting

**Family-based tests for associating haplotypes with general phenotype data: application to asthma genetics. S.**

*Horvath<sup>1</sup>, X. Xu<sup>2</sup>, S.L. Lake<sup>3</sup>, S.T. Weiss<sup>2,3</sup>, E.K. Silverman<sup>3</sup>, N.M. Laird<sup>2</sup>.* 1) Human Genetics, University of California, LA, Los Angeles, CA; 2) Harvard School of Public Health, Boston, MA; 3) Harvard Medical School, Boston, MA.

We provide a general purpose family-based testing strategy for allelic association between disease phenotypes and haplotypes when phase may be ambiguous and parental genotype data may be missing. These tests can be used in candidate gene studies with tightly linked markers. The method can be used to test for linkage and/or association between the haplotype locus and any trait influencing gene. Our proposed weighted conditional approach extends the method described in Rabinowitz and Laird [2000] and underlying the FBAT software to multiple markers. It is attractive because it provides haplotype tests for family-based studies which are efficient and robust to population admixture, phenotype distribution specification and ascertainment based on phenotypes. It can handle missing parental genotypes and/or missing phase in both offspring and parents and multiallelic or biallelic tests. This extension has been implemented in the freely available software haplotype FBAT. We used the haplotype FBAT program to test for associations between asthma phenotypes and single nucleotide polymorphisms (SNPs) in the beta-2 adrenergic receptor gene. Whereas no single SNP showed significant association with asthma diagnosis or bronchodilator responsiveness (quantitative trait), a haplotype-based global test found a highly significant association with asthma diagnosis (p-value = 0.00005) and the measure of bronchodilator responsiveness (p-value = 0.016). Download the software from [www.biostat.harvard.edu/~fbat/default.html](http://www.biostat.harvard.edu/~fbat/default.html).

**A Combinatorial Searching Method for Detecting A Set of Interacting loci Associated with Complex Traits.** *S. Zhang*<sup>1</sup>, *Q. Sha*<sup>1</sup>, *R. Cooper*<sup>2</sup>, *X. Zhu*<sup>2</sup>. 1) Department of Mathematical Sciences, Michigan Technological University, Houghton, MI 49931; 2) Department of preventive Medicine and epidemiology, Loyola University Stritch School of Medicine, Maywood IL.

Complex diseases are presumed to be the results of the interaction of several genes and environmental factors, with each gene only having a small effect to the diseases. Mapping complex disease genes therefore becomes one of the greatest challenges facing geneticists. Most current approaches of association studies essentially evaluate one marker or one gene (haplotype approach) at a time. These approaches ignore the possibility that effects of multilocus functional genetic units may play a larger role than a single-locus effect in determining trait variability. In this article, we propose a Combinatorial Searching Method (CSM) to detect a set of interacting loci (may be unlinked) that predict the complex trait. In the application of CSM, a simple filter is used to filter all the possible locus-combinations and retain the candidate locus-combinations, then a new objective function based on the cross-validation and partitions of the multi-locus genotypes are proposed to evaluate the retained locus-combinations. The locus-combination with the highest value of the objective function is the final locus-combination and the permutation procedure is used to evaluate the p-value of the test of the association between the multi-locus genotypes (or partitions of the genotypes) of the final locus-combination and the trait. The simulation studies show that the CSM has reasonable power to detect the high-order interactions of the loci even if there is no main effect. The method then applied to detect the locus-combination (among 13 loci in ACE gene) that predict systolic BP (SBP) or diastolic BP (DBP). We found that four loci with gene-gene interaction can best predict SBP with an overall p-value 3.9%, and two loci with gene-gene interaction can be best predict DBP with overall p-value 5%.

**Simultaneous estimation of haplotype frequencies, diplotype configuration, and the parameters of diplotype-based distributions of the QTL and for testing the association between QTL phenotypes and the presence of haplotypes.** *K. Shibata*<sup>1</sup>, *T. Ito*<sup>2,3</sup>, *Y. Kitamura*<sup>3</sup>, *N. Iwasaki*<sup>4</sup>, *N. Kamatani*<sup>5</sup>. 1) Department of Bioinformatics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; 2) Japan Biological Information Research Center, Japan Biological Informatics Consortium, Tokyo, Japan; 3) Mitsubishi Research Institute, Inc., Tokyo, Japan; 4) Diabetes Center, Tokyo Women's Medical University, Tokyo, Japan; 5) Division of Genomic Medicine, Department of Applied Biomedical Engineering and Science and Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan.

We have developed an algorithm, using individual genotypes at linked loci as well as their QTL phenotypes, to estimate the parameters for the distribution of QTL phenotypes for the subjects with a particular haplotype and those without it, using EM algorithm. We assumed QTL phenotype follows a normal distribution. At the same time, the algorithm calculates the maximum likelihood ( $L_{0\max}$ ) under the null hypothesis; i.e. non-association between the haplotype and QTL phenotypes, and the maximum likelihood ( $L_{\max}$ ) under the alternative hypothesis; i.e. association between the haplotype and QTL phenotypes. Then we tested the association between the haplotype and QTL phenotypes using a statistic  $-2\log(L_{0\max}/L_{\max})$ . This algorithm was implemented as a computer program QTLHAPLO. Simulation studies using SNP genotypes followed by the analysis of the simulated results by QTLHAPLO have clarified that the estimation was accurate when the linkage disequilibrium between linked loci was rather high. In such a case, the statistic  $-2\log(L_{0\max}/L_{\max})$  followed, asymptotically,  $\chi^2$  distribution with 1 degree of freedom. Empirical power using the simulated data was high enough. To be noted is that when the difference between the means of 2 different distributions are large and when the diplotype configurations of individuals are not concentrated on single events, the estimated tended to be a little higher than the real value and type I error rate was slightly higher than expected. We applied QTLHAPLO for the analysis of the real data of the genotypes at calpain 10 gene and various laboratory data concerning diabetes mellitus.

**A new strategy for investigating multilocus genotype and haplotype effects of a highly polymorphic gene: Application to the relationship between the Apolipoprotein B (*APOB*) gene and apoB plasma level.** *N. Tahri-Daizadeh*<sup>1,2</sup>, *V. Nicaud*<sup>1</sup>, *D.A. Tregouet*<sup>1</sup>, *F. Cambien*<sup>1</sup>, *L. Tiret*<sup>1</sup>. 1) INSERM U525, Hôpital Pitié-Salpêtrière, 91 Bld de l'Hôpital, 75634 Paris, France; 2) Genset-Serono Group, RN7, 91030 Evry, France.

There is increasing evidence that a detailed exploration of the whole polymorphism of candidate genes is required to obtain deeper insight into the relationship between gene variability and complex traits. The recent convergence of the massive amount of genetic data and increasing computing power offers a new opportunity to better assess the relationship between gene variability and phenotype(s). We propose a strategy for investigating the association between a highly polymorphic gene and a phenotype, by combining a multilocus genotype analysis and a haplotype analysis. The goal is to detect the most informative and parsimonious subset of polymorphisms explaining the variability of the phenotype. For multilocus genotype analysis, we developed a data-mining tool - termed DICE (Detection of Informative Combined Effects) which is a sequential forward-stepwise algorithm aimed at identifying the best subset of polymorphisms that are, either individually or in combination, associated with the phenotype. For haplotype analysis, we used our recently developed method of haplotype-phenotype association to determine, by a backward procedure, the most informative and parsimonious haplotype model. We illustrated this strategy by studying the association between a highly polymorphic candidate gene for coronary heart disease, the *APOB* gene, and plasma apoB levels in a sample of 1442 European male subjects. Twelve common bi-allelic polymorphisms located in the 5' and coding regions of the gene were considered. DICE identified the *N4311S* polymorphism as the most informative polymorphism in relation to apoB levels. The extensive haplotype analysis led to the same conclusion. DICE selected also the *E4154K* and the *T2488T* polymorphisms in a lower order of magnitude. According to our results, these 3 polymorphisms constitute the minimum subset of polymorphisms which should be investigated in further studies on the topic.

Program Nr: 2596 from 2003 ASHG Annual Meeting

**A Semi-Parametric Test for Genetic Associations With Age at Onset under Structured Populations.** *H. Chen, S. Zhang.* Mathematical Sciences, Michigan Technological Univ., Houghton, MI.

Gene mapping for complex diseases is a challenge in genetic studies. For many complex diseases, the disease genes influence the occurrence of the disease as well as the variability in their age of onset. For family-based studies, several methods have been developed to model the age of onset data. They showed that incorporating age of onset data into linkage analysis can potentially increase the statistical power for detecting linkage. In this paper, we study the effects of disease genes on the occurrence of the disease as well as the age of onset from a sample of unrelated individuals. We will consider the survival analysis model which incorporating the age of onset. As in the linkage analysis for familial disease, we have found that it will potentially increase the statistical power of detecting linkage. It is known that when the sampled data are unrelated individuals, there may present population stratification. We utilize genomic markers to control for the population stratification. Our proposed test statistic is valid regardless of the underlying genetic models that are subjected to structured population. For power comparison, our approach is compared to other case-control design methods that only consider affected cases and control group but without taking account the onset age. In most of the cases we considered, our method has higher power than that of the case-control design study.

**Haplotype case-control association analysis with related individuals.** *S. Browning*. Population Genetics, GlaxoSmithKline, Research Triangle Park, NC.

Traditional case-control association analyses require unrelated cases and controls. When related cases are available, such as following a linkage study, these methods are inefficient as only one case may be used from each family. I have developed an approach which uses all cases by accounting for familial correlation between the individuals genotypes, yielding significantly increased power.

Families containing cases typically also contain controls, however, in the presence of association, the haplotype frequencies of such controls will be biased towards case frequencies, diluting the control pool and reducing power to detect the association. Thus, I recommend using controls unrelated to the cases, which is a requirement of the method. The controls may, however, be related among themselves, for example related controls from CEPH families.

Related individuals do not each provide as much information as a single unrelated individual because some of their alleles are identical by descent. I weight each individual in such a way as to minimize the variance of the estimated haplotype frequencies. These weights may also be interpreted as the equivalent number of unrelated individuals for comparison of statistical power. For example, two full-siblings with no other genotyped relatives will each be given weight  $2/3$ , and are thus equivalent to  $2/3$  of an unrelated individual each, or 1.3 unrelated individuals in total.

I use the weights in an approximate likelihood and construct a likelihood ratio test statistic. An expectation-maximization (EM) algorithm is used to maximize the likelihood in the presence of missing genotypes and phase. The method can be applied to single markers as an allelic test or genotypic test, or to several adjacent markers as a haplotypic test, and may be applied to markers on either the X chromosome or an autosomal chromosome.

**A genotype-based association test for quantitative trait loci using family data.** *Y-J. Li, X.J. Qin, E.R. Martin.* Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC.

Several family-based tests of association have been proposed to test for association between marker alleles and a quantitative trait. These methods can detect linkage disequilibrium (LD) between the marker allele and the quantitative traits locus (QTL) using parents and offspring, or sibling data. Generally, these family-based tests are constructed by observing the correlation between a particular marker allele and the quantitative trait. They are inherently allele-based tests, and cannot be used to evaluate association between the trait and marker genotypes. In practice, it is of great interest to assess association at the genotypic level to understand how the combination of alleles in an individual may contribute to variation of the trait. Here, we extended the family-based allelic association method for quantitative traits proposed by Monks and Kaplan (2000) to test for association at the genotypic level. We conducted simulation studies to examine the power and type I error rates of our genotype-based association method. Simulations of nuclear families (parents and offspring) were conducted for a range of values for genetic parameters including the heritability of the trait, allele frequencies of both marker and QTL, degree of LD, and different genetic models. Family sizes from 1 child to 6 children were simulated. Our simulations demonstrate that the method has the correct type I error. Power of the novel genotype-based test is compared to the power of the allele-based method and shows that the relative power of the tests depends on the underlying genetic model. We are currently investigating more complicated family structures. This newly extended genotype association test will help us to dissect the influence of genotype on the quantitative trait of interest.

**Analysis of case-control studies of candidate genes in the presence of epistatic interactions.** *J. Millstein, W.J. Gauderman.* Dept Preventive Medicine, Division of Biostatistics, Univ Southern California, Los Angeles, CA.

For complex diseases, accounting for possible gene-gene interactions may prove to be an integral part of any thorough strategy designed to identify susceptibility genes among a group of candidate genes. However, the number of possible interactions can be large, and the resulting multiple testing problem cannot be ignored. We developed a search strategy to address this issue. Candidate genes are tested in stages according to the order of interaction, starting with the marginal effects of each gene. We perform likelihood ratio tests based on a logistic model. Each stage considers interactions of a given level and lower order terms. Each model is compared against a null model containing an intercept term and any lower order terms that were significant in reduced models. The number of gene sets to test for possible interactions is reduced in a preliminary step of the analysis that ranks gene sets according to a chi squared statistic based on the observed vs. expected number of subjects with variant alleles at all loci in each gene set. Only the upper ranked gene sets are selected for analysis of possible epistatic interactions. We compared strategies to find susceptibility genes, not identified by their marginal effects alone, using simulated case-control data sets. Each replicate consisted of 200 cases and 200 controls, with 20 independent diallelic genes, common variants, and a binary phenotype with 10 percent prevalence. The disease odds ratio was set to 2 for carriers of 1 variant allele at each of 3 designated loci. **Results:** Single degree-of-freedom tests of interaction with Bonferroni corrections and no gene set eliminations (an approach adopted by many) were limited in effectiveness for finding additional susceptibility genes. Our approach more than doubled the number of new genes found. Our approach was further improved by pre-screening gene sets to eliminate those least likely to influence disease risk. **Conclusions:** Accounting for possible interactions using multi degree-of-freedom tests and reducing the number of gene sets considered for interactions can significantly increase the number of susceptibility genes found in the presence of epistasis.

Program Nr: 2600 from 2003 ASHG Annual Meeting

**Case-control interval estimation of haplotypic and genotypic susceptibility.** *D.V. Zaykin<sup>1</sup>, Z. Meng<sup>1,2</sup>, S.K. Ghosh<sup>2</sup>.*  
1) Population Genetics, GlaxoSmithKline, Res Triang Prk, NC; 2) Department of Statistics, North Carolina State University, NC.

Genotypic frequencies can be directly estimated from retrospective samples of cases and controls. On the other hand, genetic susceptibility depends on the prevalence - a parameter that cannot be estimated from the observed data. We study interval estimation of susceptibility that incorporates uncertainty in prevalence values. We explore this problem from both classical and Bayesian perspective. Algebraic and numerical similarity between classical and Bayesian interval estimates allows an appealing interpretation of classical intervals as bounds for genetic susceptibility. We observe that both the asymptotic classical and Bayesian interval estimates have similar length and approximate well the exact Bayesian interval estimates. Extension from genotypic to haplotypic susceptibility intervals shows that deviations from Hardy-Weinberg equilibrium induced by the retrospective design need to be taken into account.

**Confounding from Cryptic Relatedness in Association Studies.** *B. Voight, J.K. Pritchard.* Human Genetics, Univ Chicago, Chicago, IL.

It is well known that case-control studies of association may be susceptible to false positives that result from population structure. However, it is less often recognized that cryptic relatedness among the cases is another potential source of confounding (Devlin and Roeder, 1999). The basic argument is that if a trait has a (partial) genetic basis, then individuals who share the trait are more likely to be related to one another, than are random controls, resulting in extra variance (and inappropriate significance values) of the usual chi-squared test statistic for association. Until now there has been little work to assess under what scenarios this type of confounding is likely to lead to inflated rates of false positives, in practice. In order to address this question, we have modeled the relationship between the inflation factor (IF) due to confounding,  $IF$ , and the relatedness within a case-control sample, by applying the work of Risch (1990a). We find that this inflation factor depends on the relative recurrence risk ratio,  $r$ , on the model for relatedness within the study population from which the cases and controls are drawn, and on the sample size of the study. Using a Wright-Fisher model, we simulated a randomly mating population segregating a single disease locus (parameterized by  $r$ ) and observed the relationship between  $r$  and the expected value for  $IF$ . Also, we examined the distribution of relatedness in a given study population size over time, to better understand what models of cryptic relatedness one expects to observe in a given population.

Program Nr: 2602 from 2003 ASHG Annual Meeting

**Statistical approaches for genetic linkage in affected sib-pairs in the presence of genomic imprinting.** *C.C. Wu, S. Shete, C.I. Amos.* Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX.

Genomic imprinting which exists in several mammalian genes describes the phenomenon in which the degree to which a gene expresses itself depends on its parental origin and has been demonstrated in a few genetic disorders, such as those for Prader-Willi syndrome, Angelman syndrome and Huntington disease. Here we present complete mathematical formulae for probability distributions of alleles shared identical by descent by affected sib pairs incorporating an imprinting parameter. We propose the corresponding variance components and their relationship to the increase in relative risk ratios. We discuss and compare the power and sensitivity of the usual significance tests and the tests incorporating an imprinting parameter for genetic linkage when the imprinting effect is present or absent.

**Case-Control studies adjusted for age of disease onset.** *S. Ghosh.* Applied Statistics Unit, Indian Statistical Institute, Kolkata, INDIA.

Genetic case-control studies for detecting allelic association do not take into account age of disease onset, that is, they ignore the fact that some of the individuals considered as controls may in reality possess the disease mutation and manifest the disease in the future. This can lead to erroneous estimates of allele frequencies among controls and hence, the power of the test for association can be greatly reduced. The purpose of this study is to propose an age of onset adjusted test using concepts from survival analysis. We use the Cox Proportional Hazard Model (1972) in which the frequency of a particular marker allele possessed by an individual is used as a covariate. The test for association is based on the regression coefficient linked to this covariate. An advantage of this model is that alleles at a polymorphic marker can be considered simultaneously, thereby circumventing the problem of multiple tests. Monte-Carlo simulations are performed under different disease models to evaluate the increase in power due to the age-adjustment. We find that the relative increase in power is more under dominant models than under recessive models. Under reduced penetrances, the increase becomes less pronounced.

**A bootstrap approach to SNP marker selection.** *C.T. Ekstrøm<sup>1</sup>, E. Budtz-Jørgensen<sup>2</sup>*. 1) Dept Mathematics & Physics, Royal Vet & Agricultural Univ, Denmark; 2) Dept Biostatistics, Univ of Copenhagen, Denmark.

Recent advances in molecular biology have made it realistic to create dense maps of single nucleotide polymorphisms (SNPs) that are required for identification of susceptibility genes of complex traits. However, the large number of genotyped SNPs makes it difficult to identify the SNP markers that influence the traits of interest -- especially in the presence of epistasis. Once influential SNP markers are identified they can be examined more closely through functional studies.

We propose a bootstrap approach for covariate (marker) subset selection. This method identifies markers that are likely to influence the trait of interest and the selection probabilities provide estimates of the relative importance of each SNP marker. In addition, the bootstrap approach can be combined with both deterministic and stochastic model selection schemes to infer the most likely model for the SNP markers, and the proposed method is applicable to both quantitative and qualitative traits.

A simulation study is used to assess the sensitivity and specificity of the proposed method for different genetic models. The simulations show that the bootstrap method has high sensitivity and specificity when the ratio of genotyped SNP markers to individuals is low (5) or when the effect of the susceptibility genes is high. Also, the posterior probability of importance of each markers provides a useful way to select SNP markers for further studies.

**Empirical significance of association tests of related cases and controls in isolated populations with known genealogy.** *P. Forabosco*<sup>1,2</sup>, *M. Falchi*<sup>2</sup>, *C. Cappio Borlino*<sup>2</sup>. 1) IGP-CNR, Alghero, Italy; 2) SharDna lifesciences, Cagliari, Italy.

In small isolated populations large pedigrees comprising multiple affected individuals may be reconstructed when genealogical records are available. The resulting pedigrees can be very complex with many inbreeding loops since extant individuals are usually related through multiple lines of descent. Linkage analysis of such pedigrees can be problematic and selection of specific sub-pedigrees greatly influences both the power and the false positive rate of the linkage studies. A powerful approach in these populations is LD mapping through the ascertainment of related affected individuals, which would enrich for genetic forms of multifactorial diseases and where a large proportion of affected individuals are likely to share the same disease-predisposing gene inherited from common ancestors. Although there are evident advantages to using related cases, genotype correlations among them will exist and their biological relationship must be accounted for in the statistical analysis, to avoid an increase in the type I error rate of the statistical test. New analytic methods have been proposed to overcome this problem. We propose a simulation-based method suitable for isolated populations with known genealogy that allows the simultaneous use of all the available biologically related cases and controls. Random chromosomes are simulated in the whole pedigree connecting all affected and unaffected subjects in order to estimate the background association due to relatedness among the individuals. Empirical significance of the association test is then assessed by counting the number of times a statistical test for association equal or greater than the one observed is obtained in the replicated samples. We investigated the validity of the approach and we present the results obtained performing 10,000 simulations on a 2166-individual pedigree that comprises close and remote affected and control members in the last generations. We obtained significant evidence of association under different ascertainment schemes, marker densities, frequencies and locations of the gene.

**A single shared haplotype among DM2 (CCTG)<sub>n</sub> expansion mutation carriers from different European origins indicates ancestral founder effect.** *L. Bachinski*<sup>1</sup>, *European Neuromuscular Centre DM2/PROMM Consortium*<sup>2</sup>, *F.A. Wright*<sup>4</sup>, *R. Krahe*<sup>1,3,5</sup>. 1) Section of Cancer Genetics, University of Texas M. D. Anderson Cancer Center, Houston, TX; 2) Lt.Gen. van Heutszlaan 6, 3743 JN Baarn, The Netherlands; 3) Human Cancer Genetics Program, Department of Molecular Virology, Immunology and Medical Genetics, Comprehensive Cancer Center, Ohio State University, Columbus, OH; 4) Department of Biostatistics, University of North Carolina, Chapel Hill, NC; 5) Folkhlsan Institute of Genetics, University of Helsinki, Helsinki, Finland.

Myotonic dystrophy (DM), the most common form of muscular dystrophy in adults, is collectively recognized as a clinically and genetically heterogeneous group of neuromuscular disorders. The causative mutations at two loci (DM1 and DM2) have been identified and found to be expansions of (CTG)<sub>n</sub> or (CCTG)<sub>n</sub> microsatellite repeats in chromosomes 19q13.3 and 3q21.3, respectively. In DM1 a single founding haplotype has been recognized in patients of Eurasian descent. To study the history and evolution of the DM2 mutation, we constructed a comprehensive physical map of the DM2 region around ZNF9. We studied disease chromosomes in 17 kindreds of European origin from geographically separate populations. As controls we included 108 members of 11 families and 10 unrelated individuals from matching ethnic backgrounds. High-resolution haplotype analysis with 5 microsatellite and 22 single nucleotide polymorphism (SNP) markers around the DM2 mutation identified extensive linkage disequilibrium (LD) and a single shared haplotype of at least 132 kb among patients from the different populations. With the exception of the (CCTG)<sub>n</sub> expansion mutation, the DM2 haplotype is identical to the most common haplotype in normal individuals. This situation is reminiscent of that seen in DM1. Taken together, these data suggest a single founding mutation in DM2 patients of European origin. We estimate the age of the founding haplotype and the DM2 (CCTG)<sub>n</sub> expansion mutation at approximately 5,000-12,000 years.

**A new powerful non-parametric two-stage approach for testing multiple phenotypes in family-based association**

**studies.** *C. Lange*<sup>1</sup>, *H. Lyon*<sup>3</sup>, *D. DeMeo*<sup>2</sup>, *B. Raby*<sup>2</sup>, *E.K. Silverman*<sup>2</sup>, *S.T. Weiss*<sup>2</sup>. 1) Department of Biostatistics, Harvard Department of Biostatistics, School of Public Health, 655 Huntington Avenue, Boston, MA 02115, USA; 2) Harvard Medical School, Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115, USA; 3) Division of Genetics, Children's Hospital, 300 Longwood Avenue, Boston, MA 02115, USA.

We introduce a new powerful non-parametric testing strategy for family-based association studies in which multiple quantitative traits are recorded and the phenotype with the strongest genetic component is not known prior to the analysis. In the first stage, using a population-based test based on the generalized estimating equation approach, we test all recorded phenotypes for association with the marker locus without biasing the nominal significance level of the later family-based test. In the second stage the phenotype with the smallest p-value is selected and tested by a family-based association test for association with the marker locus. This strategy is robust against population admixture and stratification and does not require any adjustment for multiple testing. We demonstrate the advantages of this testing strategy over standard methodology in a simulation study. The practical importance of our testing strategy is illustrated by application to the Childhood Asthma Management Program Asthma data sets.

**Strategies for positional cloning within regions of established linkage: case selection using IBD status on power of case-control studies.** *M.I. McCarthy*<sup>1</sup>, *J.C. Whittaker*<sup>2</sup>. 1) Wellcome Trust Ctr for Human Genetics, Oxford, UK; 2) Dept of Epidemiology and Public Health, Imperial College, London, UK.

Given a region of convincing linkage to a complex trait, identifying the aetiological variant(s) may require case-control analyses involving vast numbers of regional SNPs. The scale of such efforts demands careful sample selection to enhance power through amplifying case-control allele/haplotype frequency differences at susceptibility sites, particularly when, as with allelotyping, frequency determination is imprecise. The value of selecting cases from multiplex sibships is established: here we consider the merits of adding selection using IBD status at the linked region. For a wide range of susceptibility-locus models (varying in dominance, effect size, allele frequency), we calculated sample size ratios that compared the relative power, for case-control analyses, of case-ascertainment based exclusively on probands from ASPs sharing IBD2 in the region with that from unselected population cases (SSR1) or ASP probands regardless of IBD status (SSR2). Optionally, analyses allowed for pooling-specific error (an extra SD of 2% in frequency difference between pools). Predictably, IBD selection was most effective for recessive models with SSR1 reaching 0.04 and SSR2 0.19 (i.e. a five-fold reduction in sample size associated with IBD-selection), but benefits were substantial across all models. For multiplicative models, assuming individual typing, SSR1 ranged from 0.16-0.44, and SSR2 from 0.55-0.69; and for allelotyping, from 0.09-0.47 and 0.45-0.71. For example, given a control allele frequency of 10% and penetrance ratio of 1.5, asymptotic case frequencies are 14.3% (unselected cases), 17.2% (unselected probands) and 20% (IBD2-selected probands). Respective sample size estimates (90% power,  $\alpha=0.05$ ) are 1219, 478 and 266 for individual typing and 2098, 792, 428 for allelotyping. We conclude that, where linkage information is available and the number of samples that can be genotyped is limited, IBD-selection represents a valuable strategy for enhancing power. Also, IBD-selection provides compensation for some of the power lost through additional experimental error when allelotyping.

**Linkage disequilibrium mapping via cladistic analysis of SNP haplotypes.** *A. Morris<sup>1</sup>, C. Durrant<sup>1</sup>, K. Zondervan<sup>1</sup>, S. Hunt<sup>2</sup>, P. Deloukas<sup>2</sup>, L. Cardon<sup>1</sup>.* 1) Wellcome Trust Ctr Human Gen, Oxford, United Kingdom; 2) Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

Disease-marker association studies are recognised as having the potential to map complex disease genes. The key to this approach to mapping is linkage disequilibrium (LD) between marker SNPs and disease mutations in the same gene, generated by the recent shared ancestry of chromosomes carrying the same mutation, represented as a gene tree. Recombination breaks down LD by trimming the founder haplotype shared by chromosomes carrying the same mutation. After many generations, the founder haplotype is preserved only in the region flanking the disease gene, where a mismatch of alleles can then occur as a result of marker mutation.

Here we present a novel approach to mapping via cladistic analysis of SNP haplotypes obtained from association studies. Large genomic regions are treated as sliding windows of SNPs, with independent analyses performed within each window. Pairwise differences between haplotypes are expressed in terms of weighted marker mismatches within the window of SNPs. This metric is consistent with haplotype diversity driven by marker mutation, and corresponds to blocks of LD preserved by minimal recombination within each window.

Ignoring disease phenotype, haplotype diversity is represented by a cladogram. In windows overlapping the disease gene, the cladogram is expected to approximate the gene tree underlying the shared ancestry of sampled chromosomes. Consequently we expect correlation between disease phenotypes and clusters in the cladogram, with excess sharing of the founder SNP haplotype(s) among the high risk clade(s) of chromosomes.

Our method is developed in a logistic regression framework that can be generalised to incorporate covariates. We demonstrate the power of this approach by simulation of high density SNP data based on empirical patterns of LD across a 10Mb region of chromosome 20, highlighting substantial gains over single locus tests to detect associations for a wide range of complex disease models.

**A powerful test of linkage in the presence of association for nuclear families with arbitrary patterns of missing information.** *J.P. Lewinger*<sup>1,2</sup>, *S.B. Bull*<sup>2,3</sup>. 1) Department of Statistics, University of Toronto, Toronto, Canada; 2) Samuel Lunenfeld Research Institute, Toronto, Canada; 3) Department of Public Health Sciences, University of Toronto, Toronto, Canada.

We introduce a new test of linkage for nuclear families that overcomes some of the limitations of widely used tests such as the TDT and SDT. This linkage test uses all available family information, including unaffected siblings and homozygous parents, and it can be applied to families with any pattern of missing data. Like the TDT, it is a distribution free test, making it immune to bias due to population stratification, ascertainment, or misspecification of the genetic model. With complete parental genotypes, the distribution free property is obtained by the within-family randomization scheme proposed by Rabinowitz and Laird (Hum Hered 2000) in which the phenotypes of all individuals are kept fixed, while the children's genotypes are randomized according to the Mendelian transmission probabilities. With missing parental genotypes, a constrained randomization of the children's genotypes is required. The test statistic is a conditional likelihood ratio that yields asymptotically optimal power under the standard two point linkage model. Through a large Monte Carlo study we show that the proposed test has near optimal power for finite sample sizes, outperforming the TDT in the wide range of scenarios explored. For example, under a dominant model with incomplete penetrance, disease prevalence of less than 1% and a high level of allelic association, the proposed test achieved close to 70% power with 150 families, while the TDT had less than 10% power. Although power is reduced when parental genotypes are missing, the proposed test is always more powerful than the SDT. By randomization of the parental alleles we also construct a distribution-free and asymptotically optimal test of allelic association in the presence of linkage. However, our Monte Carlo study shows that contrary to a view held by many, testing for association in the presence of linkage is intrinsically less powerful than testing for linkage in the presence of association.

Program Nr: 2611 from 2003 ASHG Annual Meeting

**Positional Cloning by Linkage Disequilibrium.** *N. Maniatis, A. Collins, J. Gibson, N.E. Morton.* Human Genetics Division, Southampton General Hospital, Southampton University, Southampton SO16 6YD,UK.

Efforts to map genes for common diseases using parametric linkage methods have had limited success. As a result, linkage disequilibrium (LD) mapping has been the subject of much theoretical work in the last few years. Recently, LD maps were developed (Maniatis et al., 2002) which are expressed in LD units (LDU), discriminate blocks of conserved LD and have additive distances and locations monotonic with physical (kb) and genetic (cM) maps. Currently, the focus of LD research is to localize determinants of disease in the DNA sequence (positional cloning). Here we present the methodology for positional cloning using LD based on two extensive bodies of data on which currently ideas of blocks are based. The main objective is to estimate the kb location of a causal SNP as accurately as possible, with its support interval. The operating characteristics of kb and LDU maps are determined by using composite likelihood (CL) expressing regressions and correlation of SNPs. In this study we have established type I and type II errors and CL support intervals for multiple single SNPs, using simulation only for the type I error. The methodology is validated by showing that the type I error results are acceptable by the Wald theorem under all maps, metrics, and contrasts of the Malecot models. The results also shows greater power when the kb map is replaced by a map in LDU and the superiority of the regression metric. The results provide a guide to efficient positional cloning by SNPs and a benchmark against which the power of positional cloning by alternatives based on haplotypes may be measured.

**Effects of demographic histories on behavior of the linkage disequilibrium.** *S. Mano*. Department of Molecular Life Science, Tokai University School of Medicine, Isehara, Kanagawa, Japan.

For the linkage disequilibrium mapping, effects of the demographic histories of the populations to behavior of the linkage disequilibrium is critical issue. For quantitative considerations, simulation based study is one possibility, although it has its own limitations which come from sampling variability and unexpected constraints. If possible, analytical treatment is more preferable. However, in contrast to the treatment of allele frequencies, the diffusion equation method for the linkage disequilibrium is not discussed in detail, especially for the transit behavior in which we are interested, because of its complicated structure. Remarkably, Xiong and Guo solved the equation for the moments analytically by recasting the equation into simpler form with an constraint that the marker frequencies in controls to be fixed. The constraint holds for rare disease causing alleles, however, for common alleles, it might do not well. Additionally, the constraint prevent us to pursue the issue on fixation of marker alleles by the genetic drift. So, unconstrained behavior of the equation might have importance in its own right. However, because of the higher order terms of the moments in the equation, without imposing some constraints, the analytical solution seems to be unavailable. Thus, in the present study, we will discuss behavior of the linkage disequilibrium, fixation of marker alleles, and so forth with the unconstrained diffusion equation for the moments by employing a numerical solving method. We hope that the method would promote our understanding of the stochastic nature of the linkage disequilibrium.

**Properties of linkage disequilibria between multiallelic loci to detect disequilibrium induced by genetic admixture.** X. Sheng, K. Teshima, G. Sun, R. Deka, R. Chakraborty. Center for Genome Information, University of Cincinnati, Cincinnati, OH.

It is well known that genetic admixture produces linkage disequilibria (LD) among loci, the magnitude of which depends on allele frequency differences between populations contributing to the process of admixture. For loci at which there are multiple segregating alleles, several alternative measures of LD can be defined. We examined relative efficiencies of these alternative LD measures to detect admixture-induced linkage disequilibrium (AILD) immediately following an admixture process. With a computer simulation approach, we constructed admixed populations resembling African Americans and US Hispanics, by considering allele frequency data on 10 independently segregating short tandem repeat loci (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, and THO1), using the Spanish, Nigerian, and Southwestern Native Americans as the parental populations contributing to the admixture. For two scenarios of bi-parental admixtures (European and African, and European and Native American), the locus-pairs with the largest and the smallest allele frequency differences in parental populations (designated by ) were used for the analysis. With varying degrees of admixture, we show that the magnitude of AILD is larger for loci with higher values, but AILD is not a symmetric function of the proportion of admixture. The weighted average of normalized LD values of all pairwise combination of alleles ( $D_c'$ ) between two loci has the largest absolute AILD, in comparison with eight other measures of LD. However, simulations of haplotypes in an admixed population reveal that gene/haplotype diversity based measures of LD are more efficient in detecting the deviation of AILD from zero, even though their magnitudes are smaller than AILD computed from  $D_c'$ . This is caused by a comparatively larger sampling variance of  $D_c'$ . With respect to AILD immediately following admixture, the nine alternative LD measures form five equivalent groups, consistent with observations made in the literature. (Research supported by US Public Health Service Research grants).

**Microsatellite-based linkage disequilibria in relation to recombination distance: Effects of population growth, and rate and pattern of mutation.** *K. Teshima, R. Chakraborty.* Ctr Genome Information, Univ Cincinnati, Cincinnati, OH.

Stepwise mutations at microsatellite loci, together with their high rate of mutation, raise questions as to whether microsatellite-based linkage disequilibria measures decay with recombination distance, a requirement for using microsatellites for linkage disequilibrium (LD) mapping of disease genes. Using a multi-locus coalescence-based simulation model, we investigated the relationship of the weighted mean of absolute normalized LD values between all pairs of alleles at two loci, and recombination distance, by taking into account: effects of population size and past demographic history, sample size, and mutation rate and mechanism. Specifically, we studied the power of detecting significant LD at different recombination distances in the presence of the above factors. We show that with forward-backward mutations and population growth, the expected LD continues to decay with recombination distance. However, the power of detecting significant LD is affected by population history, and patterns and rates of mutations. In populations of constant sizes, detection of significant LD is easier in smaller populations. Population growth makes detection of LD more difficult. The power increases with mutation rate in constant populations, but in growing populations, markers located further than 1cM exhibit significant LD less frequently with increasing mutation rate. For multi-step stepwise mutation models, under all demographic models, power of detecting significant LD increases with average size changes of alleles caused by individual mutations. The power increases with the sample size of the study; but the relative increase of power is not appreciable for markers located 3cM or more apart. Finally, under comparable conditions, microsatellite-based LDs are easier to detect than SNP-based LDs, but in populations with a recent history of growth, these two types of markers have nearly equal powers of detecting significant LD. These theoretical predictions are consistent with data from two isolated populations, published in the literature. (Research supported by US Public Health Service Research grants).

**Model-based methods are valuable tools to identify causal genetic variants and to detect gene-environment interactions in complex diseases.** *M. Rosenberg-Bourgin, V. Chaudru, F. Demenais.* INSERM, EMI 00-06, Evry, France.

Although model-free methods are the methods of choice to map disease genes to a chromosomal region, model-based methods may be of interest to identify causal genetic variants in that region, especially when there is linkage disequilibrium (LD), and to detect gene x environment (GxE) interactions. We conducted simulations to answer to the two questions: 1) how LD can influence the power to detect GxE; 2) how ignoring GxE can affect the detection of the causal variant. Affection status, environmental factor and marker data were generated in 165 nuclear families of varying sibship size. The liability to disease was generated under a model including a common gene (G) with a small effect, a polygenic component (P) and an environmental factor (E) interacting with the gene. The proportion of total variance due to each component was : 5% for G, 15% for the GxE component, 40% for P, the remaining part being due to random environment. The disease prevalence was set to 10%. The mode of inheritance of G was either dominant or recessive with allele frequency of 0.1 or 0.5. The environmental factor was assumed to be binary with 20% frequency in the population. We generated a tightly linked SNP with varying degree of LD with that gene: no LD ( $D=0$ ), CLD ( $D=1$ ), ILD ( $D=0.5$ ). These simulated data were analyzed using the combined segregation-linkage approach based on the regressive threshold model (REGRESS program). Power of the regressive threshold model to detect GxE was between 65-82% when  $D'=1$ , 25-28% when  $D'=0.5$ , 7-13% when  $D'=0$ , depending on the characteristics of the disease gene. This power was also found to vary when modifying the strength of GxE, the frequency of E and the nature of this factor (quantitative instead of binary). Alternatively, ignoring the presence of GxE in the analysis may affect the identification of the putative causal variant. Evidence for CLD was reduced by 60-70% when ignoring GxE as compared to taking it into account, this impact being greater for a dominant gene and when E was quantitative. Use of models that take into account both LD and GxE appear to be valuable tools to disentangle the mechanisms underlying complex diseases.

Program Nr: 2616 from 2003 ASHG Annual Meeting

**Does haplotype diversity predict power for positional cloning?** *W. Zhang, A. Collins, N.E. Morton.* Human Genetics Division, University of Southampton, Southampton, UK.

Many recent studies have established that haplotype diversity in a small region is not greatly diminished when the number of markers is reduced to a smaller set of "haplotype-tagging" markers that identify the most common haplotypes. These studies are motivated by the assumption that retention of diversity assures retention of power. Using three bodies of data and three proposed measures of diversity, we cannot confirm this assumption. When the causal marker is not included, power declines precipitously as the haplotype-tagging set diminishes. Common haplotypes have especially low power when the causal marker is uncommon. These results have important implications for construction of linkage disequilibrium maps and use of haplotypes in positional cloning.

**Gain of power for detecting haplotype-phenotype associations induced by the use of missing genotypic data.** *D.A. Tregouet<sup>1</sup>, S. Escolano<sup>2</sup>, J.L. Golmard<sup>2</sup>, L. Tiret<sup>1</sup>*. 1) INSERM U525, Paris, France; 2) INSERM U436, Paris, France.

It is now widely recognized that haplotypic information can be of potential interest for investigating the genetic component underlying complex diseases. In particular, several recent works have clearly illustrated that, in the context of candidate gene association analysis performed in unrelated individuals, haplotype-based analyses can better characterize the role of gene polymorphisms in the etiology of a disease than univariate analyses. One current limitation of such haplotype analyses is that they are generally performed in the subset of individuals whose genotypic information is available for all studied polymorphisms, leading to a likely loss of power and a possible bias. While most available statistical algorithms for haplotype-based association analysis can theoretically deal with missing genotypic data, no study has investigated the impact of using these data on the estimation of the parameters characterizing the haplotype-phenotype association. Therefore, a simulation study was carried out to clarify this point in the framework of a case-control analysis where haplotypes were defined by either 4 or 6 polymorphisms. Missing genotypic data were generated according to different patterns including completely at random, and genotypic- and haplotypic-dependent missing models. Preliminary analyses carried out by use of the Stochastic-EM algorithm showed that inferring missing genotypic data during the process of the haplotype-phenotype association analysis did not introduce any bias in the estimation of haplotype frequencies nor in that of haplotype-phenotype association parameters, whatever the pattern of missing data. However, results also showed that, while not increasing the risk of detecting false haplotype effects, the use of individuals with missing genotypic data can improve the power to detect haplotype-phenotype associations. According to the sample size and the percentage of missing data, this power could even be very close to that obtained if genotypes at all polymorphisms were available for all individuals.

**A new method for computing a multipoint Bayesian posterior probability of linkage.** *M.W. Logue<sup>1</sup>, V.J. Vieland<sup>1,2</sup>.*

1) Center for Statistical Genetics Research and Division of Statistical Genetics, University of Iowa, Iowa City, IA; 2) Department of Psychiatry, University of Iowa, Iowa City, IA.

Several recent attempts to extend the Bayesian posterior probability of linkage (*PPL*) statistic to multipoint likelihoods have depended on a moving window technique to calculate a probability of a close disease gene at each location along the marker map (Wang et al. 2001, Logue et al. IN PRESS). We show that this technique results in a multipoint PPL (*MPPL*) which may perform poorly in the fine-mapping stage of a linkage study. That is, a large MPPL at a true disease gene location could be artificially depressed by the addition of more markers near the peak. In addition, the moving window MPPL represents a smoothing of the likelihood curve that tends to broaden the peak, diffusing the precision of localization. We propose, as a solution, abandoning the moving window technique in favor of a new version of the MPPL that is directly calibrated to the magnitude of the 2-point PPL. We do this by assigning each point an estimate of the PPL we would have received from a fully informative marker based on a 2-point analysis at that location with an identical model-integrated likelihood ratio at  $\ln = 0$ . These PPLs are estimated by examining 2-point PPL simulations of linked data under a variety of generating conditions (dominant/recessive, heterogeneity/homogeneity) and data structures (affected sib-pairs and nuclear pedigrees). The result is a version of the MPPL which is on the same scale as the 2-point PPL and which is not subject to deflation simply as a function of increasing marker density.

**Optimum marker spacing for QTL detection.** *N.R. London, Y. Da.* Animal Science, University of Minnesota, St. Paul, MN.

The total cost of a gene mapping project can be divided into two major components: the cost of constructing the mapping population and the cost of genotyping. Dense marker spacing requires genotyping more markers and fewer individuals, whereas large marker spacing requires genotyping more individuals and fewer markers. A model is derived to find the marker spacing for a genome scan which minimizes the total cost of a mapping project for a given statistical power. First the number of markers required to cover  $x$  Morgans of a genome was calculated as  $m = x / [-\ln(1-2r)]$ , using the Haldane map function, with  $r$  = the maximum recombination frequency between the marker and gene or QTL. Letting  $N_i$  be the number of offspring required for a desired level of power the total number of individuals to be genotyped is  $T = N_i(1 + b/F)$ , where  $F$  = the average number of individuals per family, and  $b$  = the number of parents and grandparents genotyped per family. The genotyping cost per marker,  $C_G$ , and cost of phenotyping and DNA collection of each individual,  $C_I$ , were assumed known. The total cost of the mapping project can be estimated by:  $C = mC_G T + C_I T = N_i(1 + b/F)[-x C_G / \ln(1 - 2r) + C_I]$ , where  $N_i$  is the number of individuals and usually a function of recombination frequency and type-I and type-II errors. Estimates of optimal marker spacing were obtained by minimization of the above expression with respect to  $r$ . This expression offers an approach to study optimal marker spacing for any design and any level of type I and type II error, provided that the sample size necessary for a particular level of power can be analytically expressed. Optima were found iteratively and confirmed graphically. The optimum is independent of the type I and type II error level and genome length, but these parameters affect the value at the optimum. In all cases only a single optimum existed within the realistic parameter space (marker spacing between 0-50 cM). The value of the optimum represents an estimate of the total cost of an experiment. The optimum marker spacing for a genome scan ranges from 30cM when genotyping is expensive (\$1/marker genotype) and individuals are inexpensive (\$50), to 4cM when individuals are expensive (\$2000) and genotyping inexpensive (\$0.50).

**Testing for epistasis in non-parametric linkage analysis.** *T.A. Greenwood, N.J. Schork.* Dept Psychiatry, Univ California, San Diego, La Jolla, CA.

Testing for epistasis (the interaction between two or more genes to control a single phenotype) in non-parametric family-based linkage analysis is not trivial and full of statistical and interpretational challenges. We have devised a methodology for assessing epistasis in linkage which is intuitive, flexible, and could provide greater power than many other methods. The proposed approach works with a fundamental quantity, the fraction of alleles shared identical-by-descent (IBD) by pairs of relatives, or  $\pi$ -hat. This quantity is computed as a weighted average of three probabilities: the probability that a relative pair shares 0, 1, or 2 alleles IBD. These probabilities can be computed and provided to the user by non-parametric linkage analysis software packages such as ASPEX and MERLIN. These probabilities can be used to assess evidence for epistasis by considering them jointly at combinations of loci. One can use only sibling pairs for which one of these probabilities computed for those sibling pairs is greater than some number (e.g., 0.95). This would ensure that only sibling pairs for which there is some confidence in their assigned allele sharing measures are used in the analysis. To test epistasis, one can compute log-likelihoods of joint allele sharing probabilities using a simple multinomial model. To assess significance, randomization and simulation-based tests can be used. Ultimately, however, in order to test true epistasis one must determine if there is an excess of allele sharing at two loci relative to that based on the marginal probabilities of allele sharing at each of the two loci independently. Because thousands of combinations of loci can be tested in this manner, these analyses will be exploratory and hypothesis generating. We apply our method to genotypic data obtained from a large affected sibling pair study investigating the genetic basis of autism.

**MLIP: Parallel computation of LOD scores enabling full exploration of the trait-parameter space.** *M. Govil*<sup>1</sup>, *A.M. Segre*<sup>1,2</sup>, *M.W. Logue*<sup>1</sup>, *V.J. Vieland*<sup>1,3</sup>. 1) Division of Statistical Genetics & Center for Statistical Genetics Research, University of Iowa; 2) Department of Computer Science, University of Iowa; 3) Department of Psychiatry, University of Iowa.

There is compelling evidence for complex traits that parametric linkage methods are more powerful than model-free methods. Nevertheless, fixing the trait parameters at arbitrary (wrong) values can still reduce linkage signals. In theory, the LOD score can be maximized over trait parameters, or the trait model can be treated as nuisance parameters and integrated out of a statistic such as the posterior probability of linkage (PPL). However, either of these approaches entails computing a multi-dimensional likelihood surface, which in the case of crude grid-search may involve billions of grid points. In the absence of reliable methods for approximating these surfaces, the above approaches present a significant computational challenge. MLIP (Multiprocessor LIPed) calculates two-point LOD scores by pedigree and marker over a user-specified range of disease allele frequencies, penetrances and male and female recombination fractions. Based on a well-tested program, LIPED [Ott, 1974], it uses a cluster of CPUs and relies on the NICE distributed infrastructure [Segre et al., 2002], to provide interprocessor services and communication for parallel computing. It can handle arbitrary pedigrees, including loops and large pedigrees. To enhance robustness, automatic checkpointing and fault tolerance have been built in. Results from a preliminary version of the program, for 14 dual-processor machines, show a speedup of at least 4 to 7 times over the run time on a single processor. The structure of the pedigrees and number of marker alleles were some of the factors that affected how well a parallel run performed compared to the serial. Greater speedup was observed for datasets with larger pedigrees and/or markers with more alleles. We continue to work on optimizing performance, but the current version of the program can already be used to efficiently compute LODs over the trait space. It can also be used to benchmark computationally efficient approximations to the likelihood surface as these are developed.

**Score predictions in affected sib-pair analysis - decision-making based on incomplete information.** *M. Knutsson.*  
Clinical Science, AstraZeneca R&D Molndal, Molndal, Sweden.

Linkage analysis is always based on an imperfect data set, i.e. the inheritance pattern cannot be fully traced at all positions. Furthermore, the information may display a large variation over the genome. Hence, singling out chromosomal regions for further analysis, using linkage analysis, is not a trivial task. In order to compensate for incomplete information and to account for the varying degree of information in different regions, a prediction score is derived. Using this score as a tool for guidance, true gene harbouring regions displaying nothing but noise in the original analysis may be found and labelled as promising.

The prediction score is of the same form as the NPL (nonparametric linkage) score. However, instead of performing the IBD calculations conditional on the null hypothesis of no linkage (IBD vector  $[z_0, z_1, z_2] = [0.25, 0.5, 0.25]$ ), the calculations are conditional on an alternative hypothesis ( $z_2 = 0.25$ ), estimated from the existing data. Since the outcome of complete IBD information is predicted, the gain of typing additional markers is estimated. Hence, the prediction score can help the researcher to decide whether (or not) and in which regions additional markers should be typed.

Some properties of the new score are shown and its usefulness is illustrated in an example.

**Identification of gene locations from maximum likelihood ASP linkage analysis: Are there features of the lod score curve that distinguish regions with two loci?** *E.R. Hauser, M. Bass, E.R. Martin.* Center for Human Genetics, Duke University Medical Center, Durham, NC.

In complex diseases, lod score curves can take a variety of different shapes, making the localization of disease genes under those curves very imprecise (Hauser et al. 1997, Roberts 1999). In previous simulation studies we explored the shape of the lod score curve when simulating a single disease locus for a complex trait (Bass et al. 2001). We found that, while individual lod score curves could be multimodal and support regions discontinuous, it was not the case that the gene would be located in regions of low lod score support. We have continued these simulations to explore the shape of lod score curves when two disease loci are in the same region. We used our simulation program SIMLA (available at <http://wwwchg.duke.edu/software/index.html>) that simulates pedigree data for models including both linkage and allelic association (Bass et al. 2002). To incorporate two disease loci in a region, we simulated a genetic heterogeneity model in which either locus can be a susceptibility locus in a given family. We varied the recurrence risk ratio to sibs ( $\zeta$ ) for each disease locus, the proportion of families segregating each locus, the distance between the two loci, the map density of markers, and the number of alleles for screen markers. We found that for map densities of about 10 cM, we could not reliably distinguish peaks for models with one locus from peaks for models with two loci. Furthermore, with two disease loci of similar  $\zeta$ , the maximum lod score was often midway between the two loci. In models with two loci the lod score could be deflated by as much as 35% from models with one disease locus. For maps of 5 cM or less, the lod score curves were more often multimodal suggesting the presence of two loci. We contrast the change in the lod score curve when there are two loci to the curve shape with one locus, as well as assess the effect of genotyping error on peak shape (Bass et al. 2001). These results have implications for follow-up in regions of linkage and fine-mapping using microsatellite markers.

**Parametric linkage analysis with automatic optimization of the disease model parameters.** *K. Strauch.* Institute for Medical Biometry, Informatics, and Epidemiology, University of Bonn, Bonn, Germany.

It is necessary to specify the parameters of the trait model prior to a LOD-score analysis. In many cases, the true disease model parameters are unknown; this holds especially for genetically complex traits. If the disease allele frequency and penetrances specified for the analysis are not sufficiently close to the true values, the power to detect linkage will be reduced, and the disease locus position will not be estimated correctly. For this reason, especially if complex traits are at issue, it can be helpful to maximize LOD scores with respect to the disease model parameters. This procedure is called 'MOD-score analysis' or 'maximizing the maximum LOD score' (MMLS). It is clearly an explorative method. Here, GENEHUNTER-MODSCORE is presented, which is an extension of the linkage-analysis program GENEHUNTER. It automatically maximizes the LOD score with respect to the disease allele frequency and all penetrances. Since the software is based on the Lander-Green algorithm which handles markers and trait locus separately, the inheritance-vector distribution (given all marker information) needs to be calculated only once and is stored. When evaluating the LOD score for many models (which needs to be done for MOD-score analysis), only the disease-locus likelihood must be recalculated. Since this part of the algorithm has been thoroughly optimized, it is now possible to perform a MOD-score analysis with many markers in reasonable time. The user can restrict the heterozygote penetrance(s) to lie in between the two homozygote penetrances, or turn the restriction off, e.g. to allow for overdominance. Imprinting models, where heterozygous individuals are distinguished by the parent who transmitted the mutation, and two different heterozygote penetrances are specified accordingly, can be taken into account as well. Practically, GENEHUNTER-MODSCORE first calculates the MOD score for a given set of pre-defined models. This reduces the probability to fall into a local maximum. Then, the model which yields the maximum LOD score is used as a starting point for fine maximization, which is carried out until the MOD score has converged to its maximum.

Program Nr: 2625 from 2003 ASHG Annual Meeting

**Estimation of allele frequencies with preferential amplification in a DNA-pooling study.** *H.C. Yang, C.L. Chen, C.S.J. Fann.* Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

DNA-pooling association mapping is a cost-saving method for localizing susceptible disease genes relative to the individual genotyping study. However, the prerequisite of a precise mapping in a DNA-pooling study is the accurate estimation of allele frequency. The sources of variation affecting the estimation of allele frequency in a DNA-pooling study have been discussed broadly in literatures (Sham et al., 2002; Jawaid et al., 2002; Barratt et al., 2002). We focus on the different amplification abilities of various nucleotides in this project. Hoogendoorn et al. (1999) proposed a simple adjustment to account for the unequal ability of amplification. The merit of Hoogendoorn's method is simple in concept and easy to employ. However, few theoretical results have been discussed about this method. We find that the method suffers from the estimation bias in some cases. The bias becomes intolerable especially for the small sample size. In this project, we provide the alternative estimation procedures to reduce the estimation bias. Some theoretical results can be borrowed with some modifications from the well-developed topic, ratio-type estimation. The suggested estimators are either unbiased or have smaller biases compared to Hoogendoorn's adjustment. The corresponding variances are of order  $1/n$ . Some simulation studies are conducted to assess the performance of different estimators. The results show the proposed methods produce smaller bias as expected. The improvement is helpful to obtain the precise estimation of allele frequency, in turn the precise disease-genes mapping in a DNA-pooling study.

**Evaluating performance of the methods incorporating covariates into the affected sib pair (ASP) linkage analysis.** *H.-J. Tsai*<sup>1</sup>, *D.E. Weeks*<sup>1, 2</sup>. 1) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Department of Biostatistics, University of Pittsburgh, Pittsburgh, PA.

In order to increase the power to detect the signals in the linkage analysis of complex traits, genetic heterogeneity has to be taken into account. During the past few years, several statistical methods have been proposed to deal with this issue by incorporating covariate information into the affected sib pair (ASP) analysis. However, it is still not clear the performance of these methods under different gene-environment (G x E) interactions. The methods evaluated in this study are: 1. the mixture model (Devlin et al. 2002); 2. the conditional-logistic model (Olson 1999); 3. the regression model with covariates and constraints (Greenwood and Bull 1999); 4. the maximum likelihood binomial method with accounting for covariates (Alcaïs and Abel 2001); 5. ordered subset analysis (Hauser et al. 1998); and 6. the logistic regression approach (Saccibè et al. 2001). We have written simulation programs to generate genome-wide data under various G x E models and disease models. The heterozygosity of all markers is 0.8. The disease allele frequencies are varied from 0.01 to 0.4 under different disease models. Each analyzed data set contains 100 pedigrees with at least 2 affected sibs and variable sibship size ranging from 2 to 5. We have generated 16,000 null replicates to estimate empirical significance thresholds. We then evaluated the power of the methods by generating 500 replicates under 8 disease models, each with the same G x E interaction. Power to detect the signals among the methods is varied under different disease models. We estimate the power of the methods under several G x E models by including the covariate with or without G x E interaction into the analysis.

**Incorporating uncertain phase into estimation and testing of haplotype associations.** *K.M. Burkett<sup>1</sup>, B. McNeney<sup>2</sup>, J. Graham<sup>2</sup>*. 1) McDonald Research Laboratories/The iCAPTURE Centre, St. Paul's Hospital - University of British Columbia, Vancouver, BC, Canada; 2) Department of Statistics and Actuarial Science, Simon Fraser University, Burnaby, BC, Canada.

Haplotypes of single-nucleotide polymorphisms (SNPs) may affect variation in complex traits by influencing regulation or function of susceptibility genes. Alternately, blocks of SNPs may be in linkage disequilibrium with unobserved susceptibility polymorphisms. In association studies of complex traits, regression models may be used to adjust haplotype associations for the effects of nongenetic cofactors or attributes. However, resolution of haplotype phase can be problematic because data are collected on unrelated subjects. Uncertain haplotypes can be inferred on the basis of data from single SNPs. However, subsequent analyses of disease associations must account for the uncertainty in haplotype assignment in order to avoid potential errors in interpretation. We describe a likelihood approach for estimating haplotype associations and quantifying the precision of these estimates in the presence of uncertain haplotype phase. A generalized linear model of disease penetrance accommodates quantitative and dichotomous disease phenotypes, non-genetic covariates and gene-environment interactions. An EM algorithm is implemented to find the maximum likelihood estimates of associations and haplotype frequencies. The resulting estimates and standard errors reflect uncertain haplotype phase. The method is applied to real and simulated datasets comprised of information on SNPs and non-genetic covariates.

**Multivariate quantitative trait locus regression mapping.** *S. Buyske*. Statistics Dept, Hill Ctr, Rutgers Univ, Piscataway, NJ.

Not all traits can be reduced to a univariate binary or quantitative trait. A number of conditions, including syndrome X, vascular disease, and developmental dyslexia, may best be described via several correlated quantitative traits, and so require a multivariate analysis. The three predominant methods for multivariate quantitative traits are reduction to a univariate trait (generally using principal components), multivariate extensions to the new Haseman-Elston method, and multivariate variance components.

We report on a new method of multivariate quantitative trait locus mapping that extends the regression method used in Merlin (Sham et al, 2002). Merlin's regression program uses its fast gene-flow engine to regress IBD status onto univariate trait sums and difference. This regression can be extended to a multivariate trait through a modification of Merlin. The modification combines estimators from each trait individually using the individual estimators' covariance structure. We were able to use the same simulation data as in Gorlova et al (2002), which looked at the power of extension to the new Haseman-Elston method and of multivariate variance components. Simulations show that multivariate trait regression using Merlin has superior power to these other methods. Nonetheless in simulations the type I error rate approaches the asymptotic rate only in large samples, so that use of empirical p-values is recommended.

**A nonparametric genetic association test for age-at-onset data.** *A.S. Allen<sup>1</sup>, E.R. Martin<sup>2</sup>, Y-J. Li<sup>2</sup>*. 1) Biostatistics & Bioinformatics, Duke University, Durham, NC; 2) Center for Human Genetics, Duke University, Durham, NC.

The analysis of age-at-onset (AAO) is an important tool in identifying genetic variants involve in complex diseases. Several linkage and association studies have used AAO as a quantitative trait in gene mapping with great success (Li et al. 2002). However, such analyses are complicated by the fact that age-at-onset data are subject to censoring, making standard quantitative trait methodologies suboptimal. Though methods that incorporate censoring are available, their primary focus is on modeling the age-at-onset distribution of the disease and thus make difficult-to-verify parametric or semiparametric assumptions on the process that generated the data (Hongzhe Li et. al, 1998; S. Horvath et. al, 2001). Here we propose a nonparametric framework for genetic association testing of age-at-disease onset data that explicitly accounts for censoring. This approach can be used to complement previously proposed modeling strategies by providing a way to confirm model testing results without being subject to difficult-to-verify assumptions. We formulate AAO association tests in unrelated and triad-based samples and evaluate our approach in a simulation study. We conclude with a discussion of extensions of the method to more complicated pedigree structures.

**Family-based tests of association for X-linked markers.** *R. Morris*<sup>1</sup>, *E. Martin*<sup>2</sup>, *N. Kaplan*<sup>1</sup>. 1) Biostatistics Branch, NIEHS, Res Triangle Pa, NC; 2) Center for Human Genetics, Duke University Medical Center, Durham, NC.

We propose two new family-based tests of association between an X-linked marker allele and a disease susceptibility allele that utilize families with incomplete parental-marker information. The first is a likelihood ratio test (LRT), which can be used if the sample has only families with one affected sib. If the sample contains families with more than one affected sib, then the LRT cannot be readily implemented and, therefore, we propose a TDT-like test. For both tests the E-M algorithm is used to infer missing parental data. Both tests can be parameterized to be insensitive to population stratification. Simulation results demonstrate validity and illustrate power calculations. Both tests can also be used to test for linkage in the presence of association, and therefore are competitors to the X-linked RC-TDT proposed by Horvath et al. (2000). Some results are presented comparing the power of the three tests.

**Reducing the sample size for genomewide association studies by combining evidence from multiple disease loci.**  
*B.P.C. Koeleman<sup>1</sup>, F. Dudbridge<sup>2</sup>.* 1) Department of Medical Genetics, UMCU, Utrecht, NL; 2) MRC Human Genome Mapping Project Resource Centre, Cambridge, UK.

Linkage analysis has been very successful in identifying mendelian disease genes, but has turned out to be of limited use for low risk, complex disease genes. Therefore, attention has focussed on genomewide association studies for identification of the multiple genes that are thought to underlie common disease. These studies require individual testing of a large number of markers (~100,000 or more). Consequently, the level of significance needs to be very strict to prevent inflation of type I error due to multiple testing, and large sample sizes are needed to attain sufficient power. Instead of testing each marker separately, or all simultaneously, it is more efficient to consider the combined evidence from subsets of markers. The Rank Truncated Product (RTP) combines the evidence from the  $K$  most significant markers ( $K \sim$  hypothesized number of disease loci), and has been shown to be more powerful for detecting association in complex disease models than existing methods. Here we show for different study aims and genetic models that the sample size needed to reach significance is markedly reduced for RTP compared to family-wise error or false discovery rate controlling procedures. When the aim of a study is to detect at least one disease locus with 80% power, an average of 12% (21% max) reduction in sample size is possible with RTP. Importantly, when the aim of a study is to detect at least five disease loci an average of 38% (46% max) reduction in sample size is reached. Reduction in sample size is modest when only two disease loci underlie disease, but increases significantly when more than two loci are involved in susceptibility for disease. RTP detects a set of markers of predefined size, of which at least one is associated. We show that the set of loci detected by RTP contains more true loci than competing methods, and these can be given priority according to order of individual significance in a follow-up study of much smaller size. Using this strategy with RTP, the initial sample sizes for genomewide association studies can be substantially reduced.

**Two-stage designs for fine-mapping of complex traits - a word of caution.** *J. Majewski, J. Ott.* Lab Statistical Genetics, Rockefeller Univ, New York, NY.

Non-parametric linkage studies of complex diseases generally produce broad LOD score peaks, which may be several cM away from the actual disease gene. It is of interest to refine the candidate regions at a minimum genotyping cost. For large sample sizes, fine-mapping efforts can be a considerable strain on a researchers resources. Here, we explore a two-stage design, where a collection of families is used in a genome-scan to identify candidate loci. Significant peaks are then fine-mapped with a dense marker map, but only using the families that individually show positive allele sharing within a chosen interval around the peak. Although the height of the resulting peak is not statistically meaningful, it can be expected that its location will be closer to the true locus, at a cost that is lower than that of fine-mapping the entire sample. We carry out simulations using sib-pairs and small nuclear families to determine the validity of such sub-sampling schemes. However, we find that under most conditions, selecting families according to the positive LOD scores at or near a locus of interest does not result in a refinement of the locus. In view of the simulation results, we caution against employing such two-stage designs and recommend fine-mapping the entire original sample as the most effective strategy.

**Power Study of a Novel Approach to Identifying a Minimum Candidate Gene Region in Complex Diseases.** *T.A. Thornton*<sup>1,2</sup>, *S.J. Kenealy*<sup>2,3</sup>, *J.L. Haines*<sup>2,3</sup>. 1) Program in Human Genetics, Vanderbilt Univ Med Ctr, Nashville, TN; 2) Neuroscience Grad Program, Dept of Biomedical Informatics, NLM Informatics Training Grant, Vanderbilt Univ Med Ctr; 3) Dept of Molecular Physiology and Biophysics, Vanderbilt Univ Med Ctr.

Genetic heterogeneity is a major confounding factor in the statistical analysis of complex human diseases. In linkage analysis, locus heterogeneity reduces the power to detect a true signal coming from a subset of families. A novel approach to this challenge, consensus haplotyping, was proposed by Hutcheson et al. (*Am J Med Gen* 117B: 90-96, 2003) in which locational mapping of candidate genes was combined with recombination breakpoint analysis. We have now performed simulation studies to evaluate the power of this approach.

We used the Genometric Analysis Simulation Program to simulate nuclear families with three children under a recessive disease model with 50% locus heterogeneity. A sample of 100 families was selected in which both parents were unaffected and at least two children were affected. Ten consecutive bi-allelic markers were simulated ten cM apart along with one disease locus, which was equidistant between two markers. A second disease locus was simulated to have no linkage to the first disease locus or any other marker.

We performed linkage analysis on the ten markers, identified the marker with the highest lod score, and selected those families that showed linkage to any marker within 20cM of this marker. We then performed haplotype analysis and identified which loci were shared on both haplotypes among the affected children. Our analyses found that this approach had greater than 80% power to correctly localize the disease gene within a 40cM region and over 60% power to localize the gene within a 30cM region. The simulation is being expanded to use more finely mapped markers, multiple genetic models, and various sample sizes. These results suggest that consensus haplotyping is a powerful approach toward localizing a complex disease gene given locus heterogeneity.

**Improved inference of missing parental data in a log-linear test of association by inclusion of unaffected siblings.**

*E. Rumpersaud, M.C. Speer, E.R. Martin.* Center for Human Genetics, Duke University, Durham, NC.

Candidate gene studies remain a powerful approach for studying complex diseases, but rely heavily on tests of genetic association. Weinberg et al. (1998) have developed a log-linear model for family-based association studies as a flexible likelihood-based alternative to the transmission/disequilibrium test (TDT). Like the TDT, the method uses genotype data from family triads (affected offspring and both parents), but has the advantage that the model can be generalized easily to different causal scenarios since the likelihood framework allows for inclusion of parameters to test for imprinting, maternal effects, and environmental interactions. In the situation where parental genotypes are missing, the expectation maximization (EM) algorithm is used to allow incomplete triads to contribute to the likelihood ratio test. We present an extension to this model, which incorporates additional information from the genotypes of unaffected siblings to improve assignment of incompletely typed families to theoretical mating type categories, thereby improving inference of missing parental data. We use computer simulations to evaluate type I error and power of the extended model. These simulations demonstrate the validity of the extended log-linear model under the null hypothesis of no association. We compare the power of the extended model to that of the original model, under varying levels of missing data. We examine the impact of violations of the low penetrance assumption, which is necessary for inclusion of the unaffected sibling into the model. We conclude that the proposed log-linear model will be an important tool for future candidate gene studies, particularly in the area of birth defects, where unaffected siblings often can be ascertained and where epigenetic factors such as imprinting may play a role in disease etiology.

**Genetic sampling and chiasm interference affect QTL mapping accuracy.** *J. Xu, Y. Da.* Animal Science, University of Minnesota, St.Paul, MN.

Current statistical analysis for mapping linked quantitative trait loci (QTL) relies on the assumption that chiasma interference is absent. With this assumption, a conditional analysis may be used to separate linked QTLs such that the effect of the target QTL is independent of those of linked QTLs, leading to more accurate estimation of the QTL effect and location. This type of conditional analysis is impossible when chiasma interference is present using current methods. We conducted a simulation study to evaluate the effect of genetic sampling and interference on QTL mapping accuracy. A theoretical population of over 10 million individuals were generated for seven loci without chiasma interference. The number of 10 million was chosen such that the true parameters assumed for the data simulation can be obtained reversely from the data. For computational efficiency, a sub-population of 20,000 individuals were randomly selected to represent the entire population. From this sub-population, 500 individuals were randomly selected for each of the 50 replicates in the simulation. For each replicate, pairwise recombination frequencies and interference coefficients were estimated for all seven loci, and QTL analysis was conducted for three cases: single QTL, two linked QTLs and three linked QTLs. The observed interference coefficients followed a normal distribution, and many observed interference coefficients were significantly different from zero based on t-tests (pvalue is less than 0.0001), showing that genetic sampling could result in significant observed chiasma interference even though chiasma interference in fact is absent. Ignoring chiasma interference in estimating QTL locations and effects resulted in large biases from the true parameters. When estimates of interference coefficients are included in estimating QTL locations and effects, the biases were reduced by more than half. The results show that considering chiasma interference in QTL analysis improves mapping accuracy, irrespective of whether the observed interference was true or purely due to genetic sampling, and would call for the development of QTL analysis taking into account chiasma interference.

**Evaluation of a BRCA carrier probability model with a random component for extended families. C.H.**

*Barcenas<sup>1</sup>, J. Zong<sup>2</sup>, G.M. Hosain<sup>1</sup>, L.C. Strong<sup>2</sup>, G.B. Mills<sup>3</sup>, C.I. Amos<sup>1</sup>.* 1) Department of Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, TX; 2) Department of Clinical Cancer Genetics, The University of Texas MD Anderson Cancer Center, Houston, TX; 3) Department of Molecular Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, TX.

The objectives of this study were to evaluate the accuracy and reliability of a BRCA mutation-predicting model (Antoniou et al, 2002) that allows for a residual polygene/modifier locus effect, to compare its performance with other models, and to study the impact that extended family information had upon risk estimation. We compared the performance in the prediction of BRCA1 and BRCA2 mutation status with the BRCAPRO (Duke Univ.), Myriad (I and II) and Couch (Univ. Pennsylvania) models. We generated and compared areas under receiver operator characteristics (ROC) curves for the different models from 121 pedigrees in which a family member had been previously tested for BRCA mutation by direct sequencing. Families were selected from a high-risk population from the Cancer Genetics clinics of The University of Texas MD Anderson Cancer Center. The tested individuals included 62 (51%) with breast cancer, 8 (6.6%) with ovarian cancer, and 8 males. Thirty-two (26%) families were of Ashkenazi descent, and 50% of the families had 3 or more members with breast and/or ovarian cancer. Twenty-three (19%) individuals had tested positive for BRCA1 mutation only, 15 (12%) for BRCA2 mutation only and 2 persons tested positive for both BRCA1 and BRCA2 mutations. All the models underpredicted the prevalence of BRCA1 and BRCA2 mutations in this population. The Antoniou model performed better than other models in the non-Ashkenazi families but was inferior to BRCAPRO in Ashkenazim. The extended family model performed slightly better than the model limited to second-degree family members. All models predicted BRCA1 better in the non-Ashkenazi families. The penetrance and allele frequency for BRCA1 and BRCA2 used in the Antoniou model may be low for the Ashkenazi families and may need readjustment according to clinical experience.

**Power Loss for Linkage Due to Dichotomization of Polychotomous Traits.** *J. Corbett<sup>1</sup>, C.C. Gu<sup>1</sup>, J.P. Rice<sup>1,2</sup>, T. Reich<sup>2</sup>, M.A. Province<sup>1</sup>, D.C. Rao<sup>1,2,3</sup>.* 1) Division of Biostatistics; 2) Department of Psychiatry; 3) Department of Genetics, Washington University, St. Louis, Missouri.

Some traits, while naturally polychotomous, are routinely dichotomized for genetic analysis. Dichotomization, intuitively, leads to a loss of power to detect linkage, as some phenotypic variability is discarded. To examine the magnitude of this power loss, we performed a simulation study where a trichotomous trait was simulated in a sample of 1,000 sib-pairs under various models of heritability. The study was replicated 1,000 times. Linkage analysis using a variance components method implemented in Mx was then performed on the trichotomous trait and compared with that on a dichotomized version of the trait. A comparison of the power and false positive rates of the analyses shows that power to detect linkage was increased by up to 22 percentage points simply by examining the trait as a trichotomy instead of a dichotomy. Under all models examined, the trichotomous analysis outperformed the dichotomous version. Comparable levels of false positive rates under both methods confirm that this power gain comes solely from the information in the trichotomous trait lost upon dichotomization. Thus, dichotomizing a polychotomous trait can lead to crippling power loss, especially in the case of many QTLs of small effect.

**An Integrated Approach to the Meta-Analysis of Genetic Association Studies using Mendelian Randomisation.** C. Minelli, J.R. Thompson, M.D. Tobin, K.R. Abrams, P.R. Burton. Department of Health Sciences, University of Leicester, Leicester, UK.

**BACKGROUND:**The use of genetic epidemiology to evaluate risk factors has been advocated to avoid confounding and reverse causation typical of classical epidemiology. In genetic studies a natural randomisation process occurs at conception in determining a subjects genotype (Mendelian randomisation), and unconfounded estimates of the phenotype-disease association can be obtained by combining information from gene-disease and gene-phenotype studies. **META-ANALYTICAL APPROACH:**When synthesising the evidence available, studies evaluating either gene-phenotype, gene-disease or both associations are encountered. If all the evidence regarding gene-phenotype and gene-disease associations is obtained from unrelated sources then separate meta-analyses will yield estimates of the pooled effects that can be combined to estimate the phenotype-disease relationship. Otherwise the correlation arising from studies that measure both associations must be considered. These studies allow evaluation of the interrelationships between gene, phenotype and disease and assessment of the assumptions on which Mendelian randomisation is based, in particular whether the genotype influences the disease risk only through the modification of the specific phenotype. A simple graphical approach is presented, using the example of methylene tetrahydrofolate reductase (MTHFR) gene, homocysteine and coronary heart disease for illustration. **CONCLUSIONS:**The uncertainty in the derived estimate of the phenotype-disease association is large, as it depends on the uncertainty in both estimates of genotype-phenotype and genotype-disease associations. An integrated meta-analytical approach to synthesise gene-disease and gene-phenotype evidence may not only increase precision, but also provide crucial information on the appropriateness of Mendelian randomisation. In fact, although Mendelian randomisation offers a powerful way of deriving unconfounded estimates of the phenotype-disease association, it is based on assumptions about the pathway from gene to disease, and serious biases may arise if these assumptions are not met.

**Quantifying the relationship between gene expressions and clinical traits in general pedigrees.** *Y. Lu<sup>1</sup>, P.Y. Liu<sup>1</sup>, H.W. Deng<sup>1,2,3</sup>*. 1) Osteoporosis Research Center, Creighton University, Omaha, NE; 2) Department of Biomedical Science, Creighton University, Omaha, NE; 3) Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, ChangSha, Hunan, P. R. China.

Standard methods of quantitative genetic analysis can be applied to microarray data. Treating messenger RNA transcript abundances as quantitative traits and examining their relationship with clinical traits have been pursued using analysis approach of quantitative genetics. Recent advances in microarray technology have increased interests in testing association between clinical traits and gene-expression levels by sampling subjects from multiplex pedigrees. However, use of these related subjects without accounting for their biological relationship can inflate type I error rate of statistical tests. We propose a general linear model approach for family-based microarray data analyses. Our method can accommodate covariance between relatives in the unmeasured genetic effects and model covariates of clinical importance in the mean structure of the model. A permutation test is described for situations in which families of small size were sampled. The efficacy and validity of our methods are demonstrated by using simulated data. We find that the proposed statistic has correct type I error rate, and higher power than Pearsons correlation statistic and family expression association test (FEXAT). We also develop a corresponding software for real data analyses.

**Association studies in stratified populations: correcting using genomic controls.** *D. Shmulewitz, D.A. Greenberg.*  
Div Stat Genetics, Dept Psych, Columbia Univ, and NYSPI, New York, NY.

Case-control association studies in stratified populations can result in spurious disease-marker associations if disease prevalence and marker frequencies differ in the subpopulations. Drawing subjects from different subpopulations shifts the standard  $\chi^2$  distribution rightward when testing for disease-marker associations in cases and controls, increasing the mean  $\chi^2$  and the p-value above expected. Reich and Goldstein suggested correcting the  $\chi^2$  distribution with the mean- $\chi^2$  value from unlinked markers. Devlin and Roeder suggested using the median- $\chi^2/0.456$ . We tested how well these correction factors corrected the stratification bias. We simulated two subpopulations with different disease and SNP allele frequencies (100 SNPs) and without any marker-disease association. We generated the subpopulations with a disease ratio (prevalence pop 1/prevalence pop 2) range of 0-1, mixed them, and used 45 datasets of 100 cases/controls. For each mixed population, we calculated the  $\chi^2$  statistic for disease association with each marker in all datasets. When a population showed evidence of stratification (mean  $\chi^2 > 1$  and p-value  $> 0.05$  over all datasets), we applied the two correction procedures, to test how effectively dividing by the mean or median/0.456 eliminated excess false associations. Without correction, up to 63% of the markers yielded significant associations. Using the mean  $\chi^2$  from 100 markers to correct the  $\chi^2$  values, false positives were reduced to 4.03%-4.91%, near the nominal 5% level. The median/0.456 was more conservative, with 4% or fewer significant associations. Using 60 markers, correcting by the mean resulted in 3.98%-5.56% significant associations, while the median/0.456 was anti-conservative ( $> 5\%$ ) for smaller disease ratios (0.02-0.4), but similar to the mean at ratios  $> 0.4$ . These data suggest that correcting by the mean is superior to the median/0.456 because the behavior of the mean does not change with different disease ratios. Case-control association studies in stratified populations can eliminate excess false positives by correcting the  $\chi^2$  distribution with the mean  $\chi^2$  from unlinked markers.

**Controlling false discoveries and minimizing genotyping burden in the search for disease mutations.** *E. Van den Oord.* Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, VA.

Geneticists are increasingly faced with the challenge to find a limited number of disease mutations from a very large set of possible candidates or markers. This brings along the risk of false discoveries and makes it expensive to systematically scan for disease mutations. We propose a method for designing gene-finding studies that allows researchers to control false discoveries and set true discoveries at desired proportions, while minimizing the genotyping burden. The approach is illustrated for a whole-genome LD scan assuming 500,000 SNPs. Results show that a design where earlier stages are characterized by very high false discovery rates (FDR) followed by an abrupt change to the required FDR in the final stage can result in a 50-75% reduction in genotyping. Neither sample size nor controlling the false discoveries will present major problems in whole-genome scans. Mainly depended on the proportion of true effect one wants to find, the amount of genotyping will be very large even is the study is completely designed to minimize the genotyping. The method is not restricted to a concrete study and can for instance be applied to the kind of multiple testing that occurs when a geneticist or the research community as a whole may have typed many candidate genes before a significant result is obtained. The implications of the method for general models of gene discovery are also discussed.

**On the probability that a novel variant is a disease-causing mutation.** *A. Mitchell, A. Chakravarti, D. Cutler.*  
Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

When a novel single nucleotide polymorphism (SNP) is identified in a patient and not found in a group of healthy controls, the variant is considered to be a candidate for the disease-causing mutation in that patient. Until now, no sampling theory existed for computing the probability that the novel SNP is a harmless variant. We have developed a population genetics based method for calculating a p-value for a mutation detection effort. The p-value depends upon the size of the control group, the length of sequence examined and the putative mode of inheritance. Our method can be applied to a heterozygous patient, a homozygous patient, with or without inbreeding, or to a patient who is a compound heterozygote.

For instance, we show that for complete resequencing of a 10kb candidate gene, the probability of finding a neutral variant in the patient that is not observed in 100 control chromosomes is 0.14 - 0.15. Our interpretation of these results is that a significant fraction of reported disease-causing mutations are likely to be low-frequency neutral polymorphisms. Our findings are consistent with current sequence-based polymorphism surveys. In a large resequencing study performed by our group, 39% of SNPs were seen only once in a sample of 80 chromosomes, indicating that a substantial proportion of neutral variants are likely to be rare.

**Distribution of the Coalescence Time of a Pair of Alleles for Stochastic Population Trajectories: Comparison of Fisher-Wright and O'Connell Models.** *K.A. Cyran*<sup>1, 2</sup>, *M. Kimmel*<sup>1</sup>. 1) Dept. Statistics, Rice University, Houston, TX; 2) Dept. Computer Science, Silesian Univ. of Technology, Gliwice, Poland.

The estimated values of some important parameters in population genetics (for example of the composite parameter =  $4N$ ) strongly depend on the population trajectory. The commonly used notion of effective population size, increases applicability of analytically obtained formulae to populations with changing number of members, however this approach does not introduce the stochastic elements, which play important role in small populations. On the other hand, the effective population sizes are often smaller than the observed counterparts (one of the major reasons is the bottleneck effect). Therefore, if the population has passed, in its history, through (even relatively short) period of small size, then the models describing the coalescence time in terms of deterministic effective population size, can lead to false conclusions. Stochasticity should be incorporated to obtain more realistic results. The paper discusses two ways of introducing the stochastic elements into calculation of two-allele coalescence time distribution. The first one uses classical Fisher-Wright model, where the change of the size of the population is proposed to follow the Galton-Watson branching process. The second, goes one step further, and not only uses a branching process for simulation of the population growth, but also it assumes that genealogy of the population can be described in the terms of branching processes. This latter approach has been proposed by O'Connell who then applied it to the estimation of the Mitochondrial Eve epoch. The results he obtained are biased towards long coalescence times compared to other estimations. This can be the intrinsic property of the method or just the problem of improper parameter estimation. The paper tries to compare the two models and indicate their similarities as well as conditions where their predictions are essentially different. This in turn will make easier to formulate verifiable hypotheses about experimental data describing diversity in human populations.

**Inclusion of relatives in a sample produces intra- and inter-locus association of alleles without biasing allele frequency estimates.** *H.S. Lee<sup>1</sup>, J.J. Hwang<sup>2</sup>, R. Chakraborty<sup>1</sup>*. 1) Center for Genome Information, Department of Environmental Health, University of Cincinnati, Cincinnati, OH; 2) Department of Legal Medicine, Korea University, Seoul, South Korea.

Samples of multilocus genotype data are collected for validating population genetic characteristics of markers (e.g., the assumptions of Hardy-Weinberg expectation, HWE: and Linkage equilibrium, LE) prior to their use in genetic epidemiology, DNA forensics, genetic counseling, etc. Convenient sampling strategies, the common practice of collecting such data, inadvertently may contain biological relatives. To evaluate impacts of this, we analyzed data on 155 pedigrees consisting of 882 individuals from the South Korean population, genotyped for 12 independently segregating STR loci (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, THO1, TPOX, and CSF1PO). Results show that allele frequencies at individual loci are not biased with inclusion of relatives in the sample. However, 4 of the 12 loci showed significant deviation from HWE, and 58 of the 66 pairwise combinations of loci deviated from the assumption of LE, with relatives present in the sample. In contrast, in unrelated individuals of these pedigrees, no locus deviated from HWE, and 7 pairs of loci showed deviation from LE, contributed by the rare alleles at these loci. Inclusion of relatives shifted the distribution of number of shared alleles/genotypes between pairs of individuals towards higher values, in comparison with their random expectations. By classifying individuals in increasing order of kinship categories, the observed allele/genotype sharing distributions were in accordance with their expectations under the assumptions of HWE and LE within each kinship category. Analytical results also assert the unbiasedness of allele frequency estimates for these markers in samples containing relatives. In summary, these observations provide an empirical support of the notion that the presence of relatives in a sample may cause deviations from HWE and LE, and tendencies of increased allele/genotype sharing, without biasing the allele frequency estimates. (Research supported by US Public Health Service Research grants).

**Identification of polymorphic motifs using probabilistic search algorithms.** *P. Majumder*<sup>1</sup>, *A. Basu*<sup>1</sup>, *P. Chaudhuri*<sup>2</sup>. 1) Anthropometry & Human Gen Unit, Indian Statistical Inst, Kolkata, India; 2) Theoretical Stat & Math Unit, Indian Statistical Inst, Kolkata, India.

We define a set of nucleotides at multiple polymorphic sites, not necessarily contiguous, occurring with a high frequency in a collection of aligned DNA sequences as a "polymorphic motif". The problem of finding such motifs naturally arises in case-control studies of common diseases and in molecular evolutionary genetics. Although identification of such motifs by an exhaustive search is possible, it is computationally infeasible. We have devised various probabilistic search algorithms for motif finding, whether or not the motif length ( $p$ ) is known. The objective function that is maximized, by a probabilistic search of the discrete space of all possible subsequences, is the frequency of a string of nucleotides at a subset of sites (when  $p$  is known) or most frequent string of maximum length (when  $p$  is unknown). Since for a given data set, the maximum motif frequency is inversely proportional to its length, we propose a statistical procedure to determine a cut-off point on motif-length. Optimization is performed in multiple sweep steps; updation is stochastic. The stopping rule is based on the total number of sweeps performed. To avoid the possibility of convergence to a local maximum, the concept of "elitism" is also implemented in our algorithms. Performance of our algorithms was extensively assessed using synthetic and real data sets. In synthetic data sets, in which the motifs were known, the probability of correct identification varied from 60%-100%. Computation time was the minimum when a parameter ( $c$ ) controlling the probabilistic search was set = 200. In real data sets, which pertained to human mt-HVS1 sequences from global populations, our algorithms discovered motifs, many of which agreed with those that are known to be associated with mtDNA haplogroups (e.g., M and U). We have also carried out coalescent simulations under different evolutionary scenarios to discover motifs in clades of phylogenetic trees. In the vast majority (90%-100%) of the simulated data sets, discovered motifs matched perfectly with the mutational histories of the sites included in the motifs.

**Tests of population substructure based on locus-specific genetic distance calculations between genotypic categories.** *C. Nievergelt, N. Schork.* Psychiatry, UCSD, La Jolla, CA.

Many natural populations have a structure that can be difficult to discern. Although anecdotal information about events such as the founding, migratory movements, mating patterns, and local geographical isolations associated with a population may exist, the precise effects such events have on the gene pool of the population require a more detailed empirical assessment. The detection and quantification of potential cryptic (or not obvious) substructure of populations has received considerable recent attention due to the negative impact such substructure can have on gene mapping efforts (e.g., in linkage and association studies), if ignored. We describe a procedure for assessing evidence for population substructure that is based on the simple intuition that if substructure exists, there will be subtle associations between alleles at unlinked loci. Our procedure requires genotyping a sample of individuals from a population on an appropriate number of loci (e.g., microsatellites, SNPs) and then assessing evidence for genetic distance (or general allele frequency differences) between groups of individuals based on genotypic categories at each locus in turn. Our procedure is quite general and powerful and can yield information about specific alleles that might be most useful for assessing subgroups of genetically homogenous individuals within a much larger, more heterogeneous population. We showcase the utility of our method on actual data (using the Autism Genetic Resource Exchange) and simulated data and compare it to other measures designed to detect substructure, such as the methods implemented in STRUCTURE (Pritchard et al. 2000) and tree-based methods (Bowcock et al. 1994, Mountain & Cavalli-Sforza 1997). Our results have important implications for the design and interpretation of large-scale genetic epidemiology studies of humans.

**Identifying major founder events in human diversification.** *K.K. Kidd, J.R. Kidd, A.J. Pakstis.* Dept Genetics, Yale U. School of Medicine, New Haven, CT.

Data for 50 autosomal loci (on 14 chromosomes), including 29 multiallelic haplotype systems, with a total of 465 independent common alleles are now available on 38 populations (averaging 53 individuals/pop) distributed across all major geographic regions of the world. These data yield an extremely robust framework for global population relationships with "branches" corresponding to major geographic regions indicating a series of founder events. The regional founder effects can be rank ordered in size. The one associated with expansion out of Africa is the largest (bootstrap = 99.6% of 1,000 replicants); that for the Native Americans is next largest (bootstrap = 99.7%), followed by that for Europe plus S.W. Asia (bootstrap = 93%), and the smallest for the E.Asia- Pacific-Siberia group (bootstrap = 54%). Considerable shared genetic drift also accrued between the Africa-Europe (western) populations and the (E.Asia- Pacific- Siberian-Native American populations (bootstrap >97%), undoubtedly representing drift at the forefront of the initial expansion of modern humans across central Asia. Within geographic regions our ability to define relationships varies considerably. All of Europe plus S.W. Asia and one N.W. Asian population cluster but only the Northern European populations form a meaningful subcluster (bootstrap = 69%). In contrast, within Africa substructure is significant for many relationships at bootstrap values of 94% to 100%. The two Pygmy groups, for example, form a cluster with 100% bootstrap support even though one of the groups is thought to have significant admixture with Bantu-speaking farmers. Serial subclustering of Native American populations in a North to South order is supported by bootstrap values varying in the range of 91% to 62%. The E.Asian cluster has a bootstrap value of 77% as a subcluster of a larger cluster including one Siberian group (at 75%) and two Pacific populations (at 54%). The Khanty from N.W. Asia are distinct from all geographic clusters, separated from the Africa-Europe-S.W.Asia group with a bootstrap value of 97% and from the E.Asia-Siberia- Pacific-American group with a bootstrap value of 87%. Supported in part by NIH GM57672, MH62495, AA09379, and NSF BCS991202.

**Multipoint association mapping of quantitative trait loci using genotype data.** *T. Wang*<sup>1,2</sup>, *H.J. Jacob*<sup>2</sup>. 1) Division of Biostatistics, Medical College of Wisconsin, Milwaukee, WI; 2) Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI.

Linkage disequilibrium (LD) approach has been successfully used in fine-scale mapping of disease genes. Reconstruction of haplotypes is essential in most of the current LD studies. In practice, however, only genotypes are observable in most situation. Departure from Hardy-Weinberg at some marker loci during the segregation may also hinder the effort. In this study, we develop a likelihood-based multipoint association mapping method for fine mapping of QTL using genotype data directly. Genotypic association between QTL and markers are examined without the assumption of Hardy-Weinberg equilibrium. From a sample of unrelated individuals within a homogeneous population, maximum likelihood estimation of the joint genotypic distribution of a QTL and markers as well as genetic effects of the QTL are derived based on an EM algorithm. The association between the QTL and each marker can then be calculated from the joint genotypic distribution of QTL and markers. Unlike the single-marker analysis, the method allows us using multiple linked markers simultaneously and would take into account the correlation information among markers. Therefore, it may be useful for fine mapping of QTL in some candidate regions that have been found by classical linkage analysis. The method is suitable for both experimentally designed populations (e.g., backcross,  $F_2$  and  $F_n$ ) and homogeneous natural populations. With the availability of dense single nucleotide polymorphisms (SNP) markers, it may be feasible to use SNP for fine mapping of QTL in a natural population.

Program Nr: 2649 from 2003 ASHG Annual Meeting

**Autosomal linked marker systems (SNPSTRs) and estimation of population divergence times: evaluating power, precision and accuracy using coalescent-based simulations.** *U. Ramakrishnan, J. Mountain.* Anthropological Sciences, Stanford University, Stanford, CA.

As the name implies, a SNPSTR is a linked molecular marker system: a Single Nucleotide Polymorphism (SNP) in close physical proximity to a Short Tandem Repeat (STR) polymorphism. Differing mutational properties of SNPs and STRs make SNPSTRs potentially very informative regarding human evolutionary history. Using coalescent-based simulations, we explore the statistical properties of  $F_{ST}$ , heterozygosity and linkage disequilibrium for SNPSTR data for a range of evolutionary histories including population divergence, bottlenecks and growth. Further, we explore how SNPSTRs improve our ability to estimate divergence times between two populations. We show that even for some simple population histories, these linked molecular marker systems increase statistical power (the ability to distinguish between alternate evolutionary hypotheses) as much as 70%. Our findings theoretically validate the potential benefits of these novel molecular markers.

**Departures from Hardy-Weinberg equilibrium for common disease models.** *J.K. Wittke, N.J. Cox.* Human Genetics, The University of Chicago, Chicago, IL.

Previous studies have explored using departures from Hardy-Weinberg equilibrium (DHW) for fine-mapping relatively simple, Mendelian recessive disorders (Feder et al., Nat. Genet. 1996; Nielsen et al., Am. J. Hum. Genet. 1999). It remains unclear whether DHW can be used in fine-mapping genetic variation underlying common diseases with complex inheritance since a thorough understanding of the nature of DHW under common disease models has not been undertaken. The number of individuals ( $N$ ) needed to detect DHW given a specified significance level is related to the difference in the observed and Hardy-Weinberg expected genotypic frequencies ( $\Delta$ ) (Weir, 1996). We show analytically that for dominant, recessive, and additive models,  $\Delta$  is a function of  $q$  (the overall frequency of the disease susceptibility allele in the general population) and  $r$ , the genotype relative risk. For the multiplicative models,  $\Delta$  is 0, and for a general disease model in which heterozygote penetrance is not simply a function of homozygotes risk,  $\Delta$  is a function of  $q$ ,  $r$ , and  $h$  (the heterozygote relative risk). For true control samples,  $\Delta$  is non-zero for all models (including the multiplicative model) and is a function of  $q$ ,  $r$  (risk in non-susceptible homozygotes),  $h$ , and  $K_p$ , the population prevalence of disease. We used these analytic functions to calculate  $\Delta$  and then  $N$  (assuming a significance level of 0.05) for cases and controls for a range of genetic models consistent with complex disorders ( $K_p$  ranging from 0.005 to 0.2 and ranging from 2 to 10). Results suggest that significant DHW can be expected in relatively small case-control samples over a wide range of allele frequencies and genetic models. For example, DHW can be expected in less than 500 patients for dominant or recessive models where  $r$  is as low as 2 over a range of population susceptibility allele frequencies between 0.11 and 0.80. Control samples of less than 500 individuals can also be expected to depart from Hardy-Weinberg equilibrium, particularly when population prevalence of disease is high ( $K_p=0.2$ ),  $h$  is 5 or greater, and  $q$  is between 0.1 and 0.7 for recessive models or 0.3 and 0.7 for multiplicative models.

**Clustering technique and association study for structured multilocus genotype data.** *T. Nakamura*<sup>1</sup>, *A. Shoji*<sup>2</sup>, *N. Kamatani*<sup>1,2</sup>. 1) Division of Statistical Genetics, Institute of Rheumatology, Tokyo Women's Medical University 10-22 Kawada-cho, Shinjuku-ku, Tokyo 162-0054, Japan; 2) Department of Advanced Biomedical Engineering and Science, TokyoWomens Medical University 10-22 Kawada-cho, Shinjuku-ku, Tokyo 162-0054, Japan.

One of the most important problems in case-control association studies using genotypic data is the structuring of the population. If the population is structured, false positive conclusions are easily obtained by the association study because the distribution of the statistics such as K.Pearson's chi square statistics for the test of goodness of fit is based on the assumption that the population is not structured. In the association study using multilocus genotype data, structuring of the population can be a confounding factor. If the confounding factor is observable like sex or age, it is possible to analyze the data by excluding the influences of the factors. However the structuring of the population is not directly observed for neither the genotype data nor the phenotype data. We proposed a method for analyzing structured multilocus genotype data. This method can suggest the association between the candidate locus and phenotype by the multilocus genotype data that may include the population structure. This algorithm consists of two parts, obtaining the population structure by the clustering algorithm and testing the association between the genotype and phenotype excluding population structure. As the clustering technique, we used the hierarchical clustering technique. The clusters were obtained by the hierarchical tree. As the method for testing association excluding the population structure, we used the Cochran-Mantel-Haenszel test, which allows the false positive to be decreased and power to be increased. The algorithms described in this manuscript have been implemented in a computer program POPSTRUCT. The program contains three modes, analysis mode, simulation mode and generate mode. The simulation results of false positive are shown. It is illustrated that POPSTRUCT can decrease type I error rate and increase power for structured data by the results.

**Computer simulation study of population stratification.** *G.A. Heiman*<sup>1</sup>, *D.A. Greenberg*<sup>2</sup>. 1) Dept. of Epi, Mailman Sch Public Hlth, Columbia Univ, New York, NY; 2) Div Stat Genetics, Dept of Biostatistics, Columbia University and NY State Psychiatric Institute, New York, NY.

**BACKGROUND:** The extent to which population stratification (PS) biases genetic association studies have been debated in the literature. Some believe that PS is not a serious concern (Wacholder, Rothman, and Caporaso 2002); while others believe that contradictory findings may be partially due to PS (Thomas and Witte 2002). Using computer simulations, we investigated what combinations of disease and marker allele frequencies will lead to artificial associations.

**METHODS:** We simulated disease and marker data in datasets of 100 cases and 100 controls. There was no relationship (association) between the marker and the disease. The parameters were the disease and marker allele frequencies. Generating disease allele frequencies were 0.05 for population 1 and 0.05, 0.02, 0.035 and 0.05 for population 2. Markers were biallelic with frequencies of 0.2 for population 1 and 0.9, 0.6, 0.2, and 0.1 for population 2. After the two populations were simulated, we merged each data set from population 1 with its counterpart in population 2. We simulated over 1000 data sets for each set parameters (N= 16 different combinations) and counted the number of datasets yielding evidence of association (false positives; nominally 5%).

**RESULTS:** There was no bias when EITHER the marker allele frequency or disease allele frequency was the same in both populations. For the other combinations of disease ratio and marker frequency difference, the false positive rate (i.e., artificial association) increased as the parameters increasingly differed in the two populations. False positive rates ranged from 6% to 99% when disease ratio was not 1 AND marker allele frequency differed in the populations. The false positive rate was often > 20% in those cases.

**CONCLUSION:** There is a narrow range of disease ratios and marker allele frequency differences that are completely free from bias in stratified populations.

**Informativeness of genetic markers for inference of ancestry.** *N.A. Rosenberg<sup>1</sup>, L. Li<sup>1</sup>, R. Ward<sup>2</sup>, J.K. Pritchard<sup>3</sup>.* 1) Program in Molecular & Computational Biology, University of Southern California, Los Angeles, CA, USA; 2) Institute of Biological Anthropology, University of Oxford, Oxford, UK; 3) Department of Human Genetics, University of Chicago, Chicago, IL, USA.

Inference of individual ancestry is useful in various applications, such as admixture mapping and structured-association mapping. Using information-theoretic principles, we introduce a general measure, the informativeness for assignment, applicable to any number of potential source populations, for determining the amount of information that multiallelic markers provide about individual ancestry. In a worldwide human microsatellite data set, we identify markers of highest informativeness for inference of regional ancestry and for inference of population ancestry within regions; these markers can be useful both in testing for and in controlling the influence of ancestry on case-control genetic association studies. Markers that are informative in one collection of source populations are generally informative in others. Informativeness of random dinucleotides, the most informative class of microsatellites, is 5-8 times that of random single nucleotide polymorphisms, but 2-5% of SNPs have higher informativeness than the median for dinucleotides.

**POPULATION GROUP ASSIGNMENT: EFFECTS OF MARKERS AND METHODS.** *B. Yang<sup>1</sup>, H. Zhao<sup>2</sup>, H. Kranzler<sup>3</sup>, J. Gelernter<sup>1</sup>.* 1) Dept Psychiatry Yale U School Med, New Haven, CT; 2) Dept EPH Yale U School Med, New Haven, CT; 3) U CT Health Ctr, Farmington, CT.

Population stratification is a critical issue for design and interpretation of genetic association studies. New methods offer the promise of more understanding of such stratification. However, little is known at this point about practical genotyping requirements for implementing these methods based on different marker types and characteristics. We evaluated the effects of number and efficiency of markers on accuracy of ethnic group assignment by STRUCTURE (Pritchard et al 2000) and a likelihood based method (LBM).  $F_{st}$  was used to evaluate marker efficiency. A panel of 37 markers [36 STRs and 1 SNP, FY-null] was genotyped for 934 individuals from the Northeastern US: 640 European-Americans (EA), 225 African-Americans (AA), and 69 Hispanics (Hisp). FY is known to be highly informative for resolving AA and EA populations. A bootstrap procedure of selecting increasing numbers of markers, to compare correlated and independent allele frequency models in STRUCTURE, showed no major difference in assignment accuracy for EA or AA. The Hisp subjects could not be clustered as a single subgroup. The relative assignment accuracy for STRUCTURE and LBM was evaluated by adding markers 1 by 1 up to 36 of the markers, with the order of  $F_{st}$  either descending or ascending. In EAs, LBM provided more accurate group assignment than STRUCTURE, with the FY locus included or excluded; the assignment accuracy reached over 0.98 using the 5 most efficient markers including FY, and using the 10 most efficient markers excluding FY. In AAs, STRUCTURE outperformed LBM with or without FY, and the assignment accuracy could reach over 0.98 when using the 15 most informative markers. Assignment accuracy was little affected by exclusion of FY for STRUCTURE, but dropped about 15% at the first marker for LBM. We conclude that by selection of the most informative markers, assignment accuracy of population groups could have the best results. LBM is an alternative to STRUCTURE in clustering population groups, but in contrast to STRUCTURE, its use depends on prior knowledge of allele frequencies in the populations studied.

**RNA interference-mediated silencing of *GJB2* expression.** *Y. Maeda*<sup>1</sup>, *K. Fukushima*<sup>2</sup>, *R.J.H. Smith*<sup>1</sup>. 1) Molecular Otolaryngology Research Laboratories, University of Iowa Hospitals and Clinics, Iowa City, IA; 2) Department of Otolaryngology, Okayama University Medical School, Okayama, Japan.

RNA interference (RNAi) is a process in which dsRNA suppresses the expression of homologous genes. Small interfering RNAs (siRNAs) are 21-23nt RNA duplex intermediates that trigger sequence-specific cleavage of mRNAs in mammalian cells. High suppression potency and sequence specificity make RNAi one of the promised tools for therapeutic application and analysis of gene function. Allele variants of *GJB2* account for half of prelingual autosomal recessive non-syndromic deafness, and several missense mutations in *GJB2* also cause autosomal dominant sensorineural deafness. To explore the possibility of applying RNAi technology to *GJB2* expression, we designed synthetic siRNAs targeting 4 different sites in the coding region of *GJB2* mRNA (siRNA1-4). These siRNAs were introduced into human HEK293 cells using the *Silencer* siRNA transfection kit (Ambion, Inc). Levels of endogenous *GJB2* expression were tested by a semiquantitative RT-PCR at 72 hours post transfection. Control and *GJB2*-targeting siRNAs were fluorescence-labeled using a Cy3-labeling kit (Ambion, Inc). The siRNAs were localized to the cytoplasm and resulted in specific reduction of *GJB2* mRNA levels. *GJB2* expression was suppressed by more than 80% of control levels by siRNA2, and by approximately 50% of control levels by siRNA1 and siRNA3 ( $P < 0.01$ ;  $n = 6-7$ ; ANOVA); siRNA4 was found to be ineffective ( $P > 0.01$ ). *GJB2* expression returned to control levels within 168 hrs post transfection. In mouse P19 cells, a murine construct homologous to siRNA2 induced significant reduction in levels of *Gjb2* mRNA ( $P < 0.01$ ); the construct homologous to siRNA4 exerted no significant effect on *Gjb2* expression levels ( $P > 0.05$ ). These results show that *GJB2* expression can be suppressed by siRNA and that there is a positional effect on the level of suppression that is observed. These data may provide a basis for controlling levels of *GJB2* expression *in vivo*, a requisite step in modulating the phenotype related to *GJB2* dysfunction. This research was supported by R01-DC02842 (RJHS).

**Hearing loss in Fabry disease: the effect of -galactosidase A replacement therapy.** *D. Hajioff<sup>1</sup>, S. Goodwin<sup>2</sup>, R.E. Quiney<sup>1</sup>, J. Zuckerman<sup>2</sup>, K. MacDermott<sup>2</sup>, A. Mehta<sup>3</sup>.* 1) Dept Otolaryngology, Royal Free Hospital, London, United Kingdom; 2) Clinical Trials Centre, Royal Free and University College School of Medicine, London, UK; 3) Dept Haematology, Royal Free Hospital, London, United Kingdom.

**Aims:** Fabry disease is a rare, X-linked lysosomal storage disease caused by deficiency of -galactosidase A. Hearing loss has been reported anecdotally in Fabry patients, but its nature and prevalence remain unclear. We aim to describe the nature and prevalence of hearing loss in Fabry disease, and its response to enzyme replacement therapy.

**Methods:** Fifteen male Fabry patients were enrolled in a randomized, double-blind study and received enzyme replacement therapy with agalsidase alfa or placebo for 6 months, followed by an open-label extension of 36 months thus far. Alongside this trial, an additional 8 men and 2 women have received open-label enzyme replacement for between 6 and 30 months thus far. Pure-tone audiometry, impedance audiometry, and otoacoustic emission testing were performed at 0 (baseline), 6, 18, 30 and 42 months.

**Results:** Nine patients (36%) had bilateral and 10 (40%) had unilateral high-frequency sensorineural hearing loss (SNHL). Three (12%) had unilateral middle ear effusions with conductive losses persisting beyond 6 months. Only 5 (20%) had normal hearing. The high-frequency SNHL deteriorated over the first 6 months in both placebo and active treatment groups by a median 6.3 dB ( $p < 0.0001$ , Wilcoxon matched pairs). This hearing loss subsequently improved above baseline by 1.5 dB at 18 months ( $p = 0.07$ ), 5.0 dB at 30 months ( $p = 0.006$ ), and 4.0 dB at 42 months ( $p = 0.01$ ).

**Conclusions:** Significant hearing loss, usually high-frequency sensorineural hearing loss, is a common manifestation of Fabry disease in adults. -Galactosidase A replacement therapy appears to reverse the hearing deterioration in these patients. This improvement, however, is gradual, suggesting the need for long-term enzyme replacement therapy.

**Investigation of the role of mannose-6-phosphate receptor (MPR300) in enzyme uptake and glycogen clearance in Pompe disease.** *D.S. Bali<sup>1</sup>, A.J. McVie-Wylie<sup>2</sup>, J. Dai<sup>1</sup>, Y.T. Chen<sup>1</sup>*. 1) Division of Medical Genetics, DUMC, Durham, NC; 2) Pharmacology and Toxicology, Genzyme Corporation, MA.

Pompe disease, a lysosomal glycogen storage disease resulting from acid -glucosidase (GAA) deficiency, is amenable to enzyme replacement (ERT) through receptor-mediated endocytosis. In vitro studies have established that the MPR300 is necessary for lysosomal enzyme uptake, however it is presently unclear if the same system is involved in vivo. Complete ablation of MPR300 expression in transgenic mice is lethal; therefore, we have generated viable tissue-specific MPR300 knockout (KO) mice to investigate this question. To further elucidate the role of MPR300 in recombinant human GAA (rhGAA) uptake and glycogen clearance in Pompe disease, muscle-specific MPR300 KO mice were crossed with GAA KO (Pompe) mice. These double KO mice were administered four weekly doses of 100mg/kg rhGAA and sacrificed 3 days after the last injection to examine enzyme levels and glycogen depletion in the target tissues. Age-matched Pompe mice were used as controls. Enzyme activity results demonstrated decreased enzyme uptake in the quadriceps and triceps of the double KO mice as compared to the Pompe mice (68% and 63% respectively); whereas in the heart rhGAA uptake was reduced only 11% compared to controls. Furthermore, glycogen content data indicated that glycogen clearance in the heart was similar in both the double KO mice and the Pompe mice; whereas in the skeletal muscle, the glycogen depletion was less in the double KO mice compared to the controls (12, 17 and 51% reduction in the double KO mice for the quadriceps, triceps and diaphragm respectively, compared to 59, 49 and 96% in Pompe mice). This data suggests that M6PR300 does indeed play a significant role in rhGAA enzyme uptake and glycogen clearance in skeletal muscle in vivo, whereas it is possible that the heart may employ alternative mechanisms for enzyme uptake and glycogen depletion. In conclusion, the tissue-specific MPR300 KO mice are an effective animal model to better define the mechanism of lysosomal enzyme uptake in vivo and this may lead to improvements in ERT currently available for Pompe and other lysosomal storage diseases.

**Aminoglycoside-induced ATM readthrough expression: A potential treatment for Ataxia-Telangiectasia.** C.H. Lai, H.H. Chun, S.A. Nahas, R.A. Gatti. Department of Pathology & Laboratory Medicine, David Geffen School of Medicine at UCLA, 90095-1713, Los Angeles, CA, USA.

Ataxia-Telangiectasia (A-T), an autosomal recessive disease, is characterized by lack of functional Ataxia-Telangiectasia mutated (ATM) protein in cells. A-T patients are disabled due to the gradual neurodegeneration. The genetic mutations found in A-T patients can be categorized as: 1) frameshifts, 2) abnormal splicing or 3) premature termination codons (PTCs). About 20% of A-T mutations in our patient mutation database ([http://www.benaroyaresearch.org/bri\\_investigators/atm.htm](http://www.benaroyaresearch.org/bri_investigators/atm.htm)) result in PTCs. Aminoglycosides, a group of widely used antibiotics, can induce and promote readthrough termination codons. The detailed mechanism of such readthrough is still not clear. Our objective was to establish the efficacy of aminoglycosides to induce translational readthrough of ATM PTC mutations, and thereby, produce small amounts of full-length functional ATM protein. Protein truncation test (PTT) was performed using templates containing different PTCs obtained by RT-PCR of different homozygous A-T lymphoblastoid cell lines (LCLs). Aminoglycoside-induced ATM expression was assessed by immunoprecipitation of nuclear extracts from treated A-T LCLs. Twelve A-T homozygous PTC containing LCLs indicated that readthrough expression in response to aminoglycosides was possible and was independent of the ATM PTC mutation. At comparable concentrations, Geneticin showed greater readthrough expression than Gentamicin. In preliminary *in vivo* experiments, low levels of full-length ATM protein were detected by immunoprecipitation from some A-T LCLs. Colony survival assays suggest that aminoglycoside-induced ATM may normalize radiosensitivity as well. These studies were prompted by the observation that A-T patients with some detectable ATM protein (e.g. <10% of normal levels), the progression of A-T symptoms are slower and milder. Thus, aminoglycoside-induced expression of ATM protein in A-T cells may provide a potential treatment for some A-T patients. This approach may also be applicable toward other genetic diseases that carry PTC mutations.

Program Nr: 2659 from 2003 ASHG Annual Meeting

**MPS I Management and Treatment Guidelines.** *J.E. Wraith, for the MPS I Consensus Guidelines Group.* Royal Manchester Children's Hospital, Manchester, UK.

**Overview:** Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder (LSD) caused by a deficiency of the enzyme, -L-iduronidase, which leads to the accumulation glycosaminoglycans throughout the body. Because of its rarity, affecting ~1:100,000 newborns, and phenotypic heterogeneity, clinical management of MPS I is variable.

**Objective/Methods:** A working group of 11 experts met to discuss the management and treatment of MPS I. The goal of the meeting was to share recommendations that would form the basis of published international consensus guidelines. The group included specialists in pediatrics, cardiology, ophthalmology, anesthesiology, transplantation, orthopedics, and genetics. **Results:** MPS I may present with diverse symptoms; therefore, patients often receive incorrect diagnoses until they are seen by a physician familiar with LSDs. To better delineate the systemic impact of MPS I and to establish system-specific evaluation and management guidelines for both severe and attenuated MPS I, the panel organized its recommendations around core MPS I manifestations. In both severe and attenuated MPS I, global concerns include impact on skeletal, dental, and GI systems, presence of hernias, and the need for careful evaluation before procedures requiring anesthesia. The guidelines identify individual systemic parameters for evaluation, define evaluation intervals for both severe and attenuated MPS I patients, and describe intervention and management options. Current intervention and treatment options were reviewed, including hematopoietic stem cell transplantation, Aldurazyme (laronidase) enzyme replacement therapy, and palliative care. The clinical study results of Aldurazyme and its role in MPS I management were discussed. **Conclusions:** As new treatment options aimed at reversing the disease process become available for patients with MPS I, patients will require closer monitoring and standardization of care. Patients will benefit from consensus-driven clinical practice guidelines that integrate all aspects of care. Key aspects of the consensus guidelines will be presented. (Sponsored by BioMarin/Genzyme LLC).

**Effects of Aldurazyme (laronidase) on Joint Mobility in MPS I.** *M. Bajbouj*<sup>1</sup>, *M. Beck*<sup>1</sup>, *J.E. Wraith*<sup>2</sup>, *L.A. Clarke*<sup>3</sup>, *E.H. Kolodny*<sup>4</sup>, *G.M. Pastores*<sup>4</sup>, *J. Muenzer*<sup>5</sup>. 1) U Mainz, Mainz, GER; 2) U Manchester, Manchester, UK; 3) UBC, Vancouver, CA; 4) NYU, NY, NY; 5) UNC, Chapel Hill, NC.

**Objective:** To evaluate the effects of Aldurazyme (recombinant human alpha-L-iduronidase, rhIDU) on joint mobility, a major source of morbidity in MPS I.

**Methods:** Maximal active joint range of motion in the knee and shoulder was assessed in 45 patients participating in a 26-wk Phase 3 placebo-controlled study followed by an ongoing open-label extension study of Aldurazyme. Within-group changes were summarized after 48 wks (Placebo/rhIDU patients, N=23) and 74 wks (rhIDU/rhIDU patients, N=22) of treatment with Aldurazyme.

**Results:** Overall changes were small and variable. In patients who had more severe joint restriction at baseline (below median), range of motion improved in all movements assessed. Changes in knee mobility were greater in rhIDU/rhIDU patients who were treated for a longer period of time.

Joint Motion	Placebo/rhIDU	rhIDU/rhIDU
	Degrees	Degrees
Shoulder Flexion Mean	15.2 (n=12)	15.4 (n=9)
Shoulder Extension L/R	12.6 (n=8) / 8.1 (n=8)	11.9 (n=9) / 15.8 (n=12)
Knee Flexion L/R	6.6 (n=7) / 3.1 (n=7)	16.0 (n=14) / 17.8 (n=13)
Knee Extension L/R	2.0 (n=12) / 3.6 (n=12)	11.6 (n=9) / 11.3 (n=9)

**Conclusion:** Improvements in joint mobility likely contribute to the functional improvements seen in MPS I patients treated with Aldurazyme. (Sponsored by BioMarin/Genzyme LLC).

Program Nr: 2661 from 2003 ASHG Annual Meeting

**Mutation-Independent Gene Therapy Strategy for Osteogenesis Imperfecta.** *H.P. McMahon, D. Allen, P. Humphries, P.F. Kenna, S. Millington-Ward, G.J. Farrar, Ocular Genetics Unit. Genetics, Trinity College Dublin, Dublin, Leinster, Ireland.*

Osteogenesis Imperfecta (OI) is a brittle bone disorder, usually caused by the presence of mutant COL1A1 or COL1A2. To date over 150 and 80 different mutations in the COL1A1 and COL1A2 genes respectively have been characterised (1). In this gene therapy study a mutation-independent suppression and replacement strategy (2) for COL1A1-linked OI was evaluated using small interfering RNAs (siRNAs). siRNA can elicit sequence dependent gene-silencing in mammalian cells (3). In this study H1-driven COL1A1 targeting siRNAs have been assessed in cells expressing COL1A1 using real-time RT-PCR. COL1A1 expression levels were reduced by up to 98%.

**Hammerhead ribozymes targeting glycine substitutions in type 1 collagen for the therapy of Osteogenesis imperfecta.** *Y. Smicun<sup>1</sup>, M.W. Kilpatrick<sup>1</sup>, D. Basel<sup>1</sup>, R.J. Wenstrup<sup>2</sup>*. 1) Dept Pediatrics, UConn Health Center, Farmington, CT; 2) Cincinnati Childrens Hospital Research Foundation, Cincinnati, OH.

Hammerhead ribozymes are small catalytic RNA molecules capable of cleaving RNA in a sequence-dependent manner. Osteogenesis imperfecta (OI) is a genetic connective tissue disorder in which mutant collagen type I can participate in but not sustain the formation of the triple helix, thus depleting the extracellular matrix of structurally intact molecules necessary for normal fibrillogenesis. Selective ablation of mutant collagen transcript could reverse the dominant negative effect exerted by the abnormal protein. In earlier studies we have shown ribozyme can efficiently abolish the mutant transcript in a model of OI derived from the pMG155 mutant Col1A1 minigene. However, as most OI phenotypes involve substitutions for triple helical glycine residues, we have tested the efficacy of ribozymes in the specific targeting of such mutations. A cDNA segment encoding the triple helical domain of the Col1A2 gene was inserted into pMG155 to produce pMG155A2. Three separate single nucleotide substitutions were introduced into pMG155A2 to specifically mutate three glycines. Mutant specific ribozymes were produced targeting each of the glycine substitutions. Gly(A)Rz targets a G to C mutation that produces a cuc cleavage site, Gly(B)Rz a G to C mutation that produces an auc cleavage site and Gly(C)Rz a G to T mutation that produces a guc cleavage site. These three ribozymes test mutations in both the first and second bases of a glycine codon, and target three different cleavage triplets. Ribozymes Gly(A)Rz and Gly(C)Rz, cleaved their target mutant transcripts *in vitro*. The ribozymes were highly specific in that neither cleaved the wild type transcript that differed from the mutant by only a single base. Production of cell lines and transgenic mice expressing these mutations and ribozymes will allow testing of the efficacy of ribozymes targeted to glycine substitutions *in vivo*. The ability to specifically target glycine substitutions that comprise the great majority of OI mutations observed further emphasizes the potential of hammerhead ribozymes in the therapy of this disorder.

**Intravenous administration of lentiviral vector, or adeno-associated virus (AAV) vector, produces metabolic and phenotypic correction of Hurler syndrome in mice.** *C.B. Whitley<sup>1</sup>, D. Pan<sup>1</sup>, S.D. Hartung<sup>1</sup>, J.L. Frandsen<sup>1</sup>, R. Gunther<sup>1</sup>, W.C. Low<sup>1</sup>, S.U. Walkley<sup>2</sup>, R.S. McIvor<sup>1</sup>.* 1) Gene Therapy Program, University of Minnesota Medical School, Minneapolis, MN; 2) Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

Mucopolysaccharidosis type I is an autosomal recessive lysosomal storage disease resulting from deficiency of alpha-L-iduronidase (IDUA) and the consequent systemic accumulation of glycosaminoglycans (GAG) heparan sulfate and dermatan sulfate. The murine IDUA knock-out model is found to be a bona fide model of Hurler syndrome, the most severe clinical phenotype associated with the correspondingly severe human molecular genetic abnormalities (e.g., W402X, Q70X). To evaluate the efficacy of gene therapy for this disorder, we constructed lentiviral and AAV vectors expressing the human IDUA cDNA from a PGK, or a CAGS promotor, respectively. Following a single intravenous administration of either of the two vectors into newborn mice, we found virtually complete metabolic correction in some individuals. Throughout the 3- to 5-month period of observation, plasma levels of IDUA enzyme were increased, in some individuals reaching levels more than 10-fold above normal. Optimal treatment resulted in normal morphology of cells in liver and other organs, including the brain. Treated mice also showed reduced levels of accumulation of secondary glycosphingolipid storage products (GM2 and GM3 gangliosides) in neurons of the hippocampus and other brain regions. Notably, treated mice performed better, with near-normal results, in behavioral tests of habituation. Quantitative PCR determinations in each tissue are facilitating a quantitative comparison of the biodistribution, and relative gene expression, of lentiviral and AAV vectors. These factors will determine the minimal requisite conditions for treatment of Hurler syndrome by gene therapy.

**A preliminary study : Can donepezil improve quality of life of Down syndrome patients?** *T. Kondoh<sup>1</sup>, N. Amamoto<sup>1</sup>, T. Doi<sup>1</sup>, H. Hamada<sup>1</sup>, Y. Ogawa<sup>2</sup>, M. Nakashima<sup>3</sup>, H. Sasaki<sup>3</sup>, K. Aikawa<sup>4</sup>, M. Aoki<sup>4</sup>, K. Tanaka<sup>5</sup>, J. Harada<sup>6</sup>, H. Moriuchi<sup>1</sup>.* 1) Dept Pediatrics, Nagasaki Univ Sch Medicine, Nagasaki, Japan; 2) Dept Radiol, Nagasaki Univ Sch Med, Nagasaki, Japan; 3) Dept Hosp Pharm, Nagasaki Univ Sch Med, Nagasaki, Japan; 4) School for the Mentally Retarded, Faculty Education, Nagasaki University, Nagasaki, Japan; 5) Kakunan Yougo Gakko, Nagasaki, Japan; 6) Faculty of Education, Nagasaki University, Nagasaki, Japan.

Most Down syndrome (DS) patients develop neuro-pathological changes at age 35 or more as seen in Alzheimer disease (AD) patients. We here report the preliminary results of donepezil, an anti-AD drug, effects on QOL of DS patients. After approved by the Ethical Review Committee, we started the 24-week double-blind study with 10 otherwise healthy DS patients. The dose was started at 3 mg per day for one week, and increased to 5 mg per day. We assessed their QOL by the questionnaire and by adaptive behavior scale (ABS) examination. As adverse effects, some patients in the donepezil (D) group had moderate side effects. But those symptoms disappeared by reducing the dose. As efficacy, four of five DS patients in the D group got the impression that their childrens QOL got markedly improved. However, the results of F test of ABS before and after this trial did not show any differences between the D group and placebo group. Our first study had two implications. First, DS patients may be more sensitive to donepezil than other individuals. Second, improvement of QOL upon donepezil therapy may not be easily shown by ordinary ABS. To address these issues, we are undergoing the next study. We examined the serum concentrations of donepezil in 6 DS patients. Our results suggest that 3 mg per day is appropriate for most DS patients, and dose-up to 5 mg per day should be done only if needed, under monitoring of blood levels. To assess QOL more sensitively and objectively, we are modifying the ABS. Furthermore, we performed brain SPECT study for 6 DS patients before the donepezil therapy. Some patients showed the brain perfusion abnormalities. We will purchase this change. In conclusion, our preliminary study suggests donepezil may help some DS patients improve their QOL.

**Pregnancy-associated fetal progenitor/stem cells differentiate into various epithelial tissues in maternal organs.**

*P.K. Khosrotehrani<sup>1</sup>, K.L. Johnson<sup>1</sup>, D.H. Cha<sup>1</sup>, R. Salomon<sup>2</sup>, D.W. Bianchi<sup>1</sup>*. 1) Division of Genetics, Department of Pediatrics, Tufts-New England Medical Center, Boston, MA; 2) Department of Pathology, Tufts-New England Medical Center, Boston, MA.

**Purpose/Methods.** Fetal hematopoietic stem/progenitor cells enter the maternal circulation during pregnancy and can persist for decades (1,2). The morphology of these cells, when analyzed in tissue sections, suggests that some of them have differentiated into other cell types (3). In the present study, we performed fluorescence in situ hybridization on archived tissue specimens from eleven women with prior male pregnancies, to localize male microchimeric cells, and identified their phenotype with immunolabeling techniques to assess their capacity to differentiate. We used antibodies to cytokeratin as a marker of epithelial cells, CD45 as a hematopoietic marker, and heppar-1 as a hepatocyte marker.

**Results.** In epithelial tissues such as thyroid, cervix, intestine or gallbladder, 14-60% of fetal microchimeric cells differentiated into cells that express epithelial markers. In addition, 4% of microchimeric cells in liver parenchyma showed evidence of hepatocytic differentiation. Conversely, in hematopoietic organs such as lymph node and spleen, 90% of the cells retained a hematopoietic phenotype, as shown by the expression of CD45. Immunostaining and histologic assessment of the phenotype of microchimeric cells was highly concordant. Differentiation of the microchimeric cells occurs preferentially in areas of disease compared to healthy tissue ( $P < 0.0001$ ).

**Conclusions.** Pregnancy associated fetal progenitor cells (PAPCs) have the ability to differentiate into hepatocytes and epithelial cells. Further studies of naturally occurring fetal progenitor cells may be useful in understanding the influence of fetal microchimerism on maternal health.

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**Adrenal cortical regeneration: murine models for tissue engineering.** *C. Truong*<sup>1</sup>, *M. Lam*<sup>2</sup>, *E.R.B. McCabe*<sup>3,4,5</sup>, *J. Dunn*<sup>1,2,5</sup>. 1) Dept Bioeng, Henry Samueli Sch Engr Applied Sci, UCLA, LA, CA; 2) Dept Surg, David Geffen Sch Med at UCLA, LA, CA; 3) Dept Peds, David Geffen Sch Med at UCLA, LA, CA; 4) Dept Hum Gen, David Geffen Sch Med at UCLA, LA, CA; 5) Mattel Children's Hospital at UCLA, LA, CA.

The adrenal cortex is a multi-layered organ that shows properties of renewal and regeneration consistent with the presence of adrenal cortical stem cells. Patients with inborn errors of adrenal development benefit from tissue engineering strategies. We examined potential models for investigation of adrenal cortical regeneration: adrenal autotransplantation and cell transplantation of freshly harvested adrenal cortical cells. In adrenal autotransplantation, right adrenal glands were excised from balb/c mice and transplanted into ipsilateral kidney under the renal capsule. Mice were sacrificed at weekly intervals, and kidneys were harvested, sectioned and stained with Hematoxylin and Eosin (H & E). One week after adrenal autotransplantation various zones of adrenal gland could be identified. By the second week, the adrenal gland became less densely packed, cell death became evident, and different zones became more difficult to distinguish. By week 3, the adrenal gland had increasing empty pore spaces occupying the gland. New cells and cell proliferation in empty spaces were evident. Our second approach involved harvesting of adrenocortical cells and transplanting them beneath kidney capsule of mice. Transplanted cells were confined within a space, using a small polycarbonate ring to create a virtual space beneath the renal capsule so growth and vascularization of transplanted cells could be studied. Transplanted mice were sacrificed 4-8 weeks post-transplantation, and kidneys were harvested, sectioned and stained with H&E. Eight weeks post-transplantation, a pale yellow tissue was visible within the rings with H&E staining consistent with adrenal cortical cells. We conclude that we have two model systems for investigating the regenerative potential of adrenal cortex. These investigations will permit identification of putative stem cells and will provide a foundation for future tissue engineering of adrenal cortex.

**Aldurazyme (laronidase) Enzyme Replacement Therapy for MPS I: 48-Week Extension Data.** *L.A. Clarke<sup>1</sup>, J.E. Wraith<sup>2</sup>, M. Beck<sup>3</sup>, E.H. Kolodny<sup>4</sup>, G.M. Pastores<sup>4</sup>, J. Muenzer<sup>5</sup>*. 1) UBC, Vancouver, Canada; 2) U Manchester, Manchester, UK; 3) U Mainz, Mainz, GER; 4) NYU, NY, NY; 5) UNC, Chapel Hill, NC.

**Objective:** To evaluate the long-term efficacy of Aldurazyme (recombinant human -L-iduronidase, rhIDU) for the treatment of MPS I. **Methods:** All patients from the 26-week Phase 3 randomized, double-blind, placebo-controlled study were enrolled in the Phase 3 open-label extension study. At Week 48 of the extension study, changes in forced vital capacity (FVC) and the 6-minute walk test (6MWT) were evaluated from baseline (start of double-blind study) or entry (start of open-label study) based on initial treatment group. Percent of predicted normal FVC was calculated by two methods: using baseline height to eliminate the confounding effects of rhIDU on posture and subsequent height measurements, or conventionally, using current heights at the time of FVC assessments. Patients receiving rhIDU throughout both phases of the study (N=22; total rhIDU exposure=74 weeks) were designated rhIDU/rhIDU, while those receiving rhIDU after cross-over from placebo (N=23; total rhIDU exposure=48 weeks) were designated Pl/rhIDU. **Results:** At Week 48 of the extension, rhIDU prevented further disease progression or lead to improvements in FVC from baseline or entry. Percent of predicted normal FVC calculated using baseline height improved by a mean of 6.6 percentage points in rhIDU/rhIDU patients after 74 weeks (p=0.001) and by a mean of 3.0 percentage points in Pl/rhIDU patients after 48 weeks (p=0.047). Measurements of FVC using current heights revealed that rhIDU prevented further disease progression in patients in both treatment groups with stabilization from baseline or entry despite anticipated decline in this patient population. At Week 48, patients in both groups continued to improve in the 6MWT with rhIDU/rhIDU patients showing a mean increase of 43.9 m (p=0.011) and Pl/rhIDU patients showing a mean increase of 37.6 m (p=0.046). **Conclusions:** This study demonstrates that long-term treatment of MPS I patients, many of whom had considerable pre-existing disease, with Aldurazyme leads to sustained improvements in clinically relevant endpoints. (Sponsored by BioMarin/Genzyme LLC).

**Receptor Function and Expression in Macrophages from Acid Sphingomyelinase Deficient Mice.** *R. Dhami, E. Schuchman.* Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY.

The acid sphingomyelinase (ASM) deficient forms of Niemann-Pick disease (NPD) are characterized by progressive accumulation of lipid-laden macrophages in many visceral organs, including the lung. Progressive accumulation of such macrophages was observed in the airways of acid sphingomyelinase deficient knock-out (ASMKO) mice, and this was attributed to elevated expression of the chemokines, macrophage inflammatory protein-2 and 1alpha. To investigate the mechanism(s) underlying enzyme replacement therapy for NPD, the uptake of rhASM by alveolar macrophages from the ASMKO mice was studied. Although the recombinant enzyme could bind to ASMKO macrophages, it could not be internalized efficiently. This was attributed to a defect in mannose-6-phosphate receptor trafficking and function. In contrast, the function and expression of mannose receptors appeared to be normal in ASMKO cells, consistent with the fact that a high mannose form of rhASM was taken up better by ASMKO macrophages than the complex form. However, even this enzyme form was internalized poorly by the ASM macrophages when compared to normal. These findings have important implications for the treatment of interstitial lung disease in ASM-deficient NPD patients, and suggest that the delivery of rhASM to cells promoting chemotaxis, rather than the airway macrophages themselves, may be the most effective approach.

**Aldurazyme (laronidase) Enzyme Replacement Therapy in MPS I: Preliminary Safety Data in Children Less Than 5 Years of Age.** *N. Guffon*<sup>1</sup>, *J.E. Wraith*<sup>2</sup>, *T. Braakman*<sup>3</sup>. 1) Hopital E. Herriot, Lyon, France; 2) Willink Biochemical Genetics Unit, Royal Manchester Children's Hospital, Manchester, UK; 3) Pharmacovigilance Dept., Genzyme Corp., Naarden, The Netherlands.

**Objective:** To evaluate safety and efficacy of Aldurazyme use in young children (<5 years) ineligible for hematopoietic stem cell transplantation.

**Methods:** Approximately 20 MPS I patients less than 5 years old are enrolling in a Phase 2 open-label study assessing Aldurazyme at 100 U/kg (0.58 mg/kg) once weekly for 12 months. It is anticipated that most of these patient will present the most severe MPS I phenotype. Safety monitoring will include physical examination, vital signs, ECG, clinical laboratory testing, immunogenicity testing, and adverse event monitoring. Investigators are requested to report all serious adverse events (SAEs) and infusion-associated reactions (IARs: related AEs on the day of infusion) to the Pharmacovigilance Department. Efficacy will be evaluated by assessing liver volume, urinary glucosaminoglycans (GAG) excretion, upper respiratory care requirements, sleep apnea by polysomnography, hearing, vision, growth velocity, electrocardiogram, echocardiogram.

**Results:** Study is ongoing. To date 11 patients have received 9-27 infusions (median 16). All patients had a Port-a-Cath placement to ease the infusion.

**Conclusion:** At submission date, conclusions concerning the study can not be made. Preliminary information on the safety and efficacy endpoints (urinary GAG) will be available at the time of the meeting. (Sponsored by BioMarin/Genzyme LLC).

**The One Year Experience of Enzyme Replacement Therapy for Mucopolysaccharidosis II (Hunter syndrome).** *J. Muenzer*<sup>1</sup>, *M. Calikoglu*<sup>1</sup>, *D. Towle*<sup>1</sup>, *S. McCandless*<sup>1</sup>, *A. Kimura*<sup>2</sup>. 1) Dept Pediatrics, Univ North Carolina, Chapel Hill, NC; 2) Transkaryotic Therapies (TKT), Inc, Cambridge, MA.

Mucopolysaccharidosis II (MPS II) is an X-linked recessive disorder caused by the deficiency of the lysosomal enzyme iduronate-2-sulfatase (I2S). There is no effective therapy for MPS II. The goal of this study is to evaluate the safety and clinical activity of I2S in the treatment of MPS II. Human I2S was produced by genetic engineering technology in a human cell line by TKT. The phase I/II clinical trial was a randomized, double-blind, placebo-controlled clinical study, which enrolled 12 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 (3 patients) or placebo (one patient) by IV infusions biweekly for 6 months. All 12 patients have successfully completed the 6-month double-blind study and all elected to enroll in the open-label extension study. In the extension study, patients have currently received 6 months of open-label enzyme at the same dose, with 9 patients (3 at each I2S dosage level) receiving a total of one year of therapy. Urinary glycosaminoglycan excretion was consistently reduced at 50% and 45% after 6 months and one year of I2S treatment, respectively. Liver and spleen volumes were also reduced 27% and 26%, respectively, after one year of therapy. In addition, improvements in the 6-minute walk test (13% and 28% in the 0.5 and 1.5 mg/kg groups, respectively) and joint range of motion (4 to 7 joint motions improved in 7 of 9 patients) were observed after one year of treatment. Infusion-related reactions have occurred in 8 patients and have been successfully managed by slowing the infusion rate and using premedications. IgG antibodies developed in one patient receiving the 1.5 mg/kg dose during the first six months. An additional 5 patients in the 0.5 and 1.5 mg/kg dose groups developed IgG antibodies after 12 months. The development of low-titer antibodies appears not to have any clinical consequences. These results indicate that the I2S preparation is clinically active and is a promising treatment for somatic disease in MPS II.

**An *in vivo* comparison of the efficacy of acid -glucosidase produced in CHO cells and transgenic rabbits.** A. McVie-Wylie<sup>1</sup>, C. Rogers<sup>1</sup>, R. Gotschall<sup>2</sup>, B. Thurberg<sup>1</sup>, W. Canfield<sup>2</sup>, R. Mattaliano<sup>1</sup>, L. Andrews<sup>1</sup>. 1) Genzyme Corporation, Framingham, MA; 2) GRI, Genzyme Corporation, Oklahoma City, OK.

Pompe disease is an autosomal recessive disorder of glycogen metabolism resulting from the deficiency of the lysosomal enzyme acid -glycosidase (GAA). Enzyme replacement therapy (ERT) has been developed for Pompe disease and early phase clinical trials have been conducted, or are ongoing, using recombinant human GAA purified from the milk of transgenic rabbits (tgGAA) and from genetically modified CHO cells (rhGAA). In independent laboratory studies, both enzymes have demonstrated efficacy in the Pompe mouse animal model; however, for direct comparison, a study was designed to determine the relative efficacy under the same experimental conditions. Pompe mice were administered either tgGAA or rhGAA intravenously at three dose levels (20, 60 and 100mg/kg) weekly for 4 doses, and sacrificed 24 hours after the last injection. Results demonstrate that under the conditions of the study, and assays performed, a dose dependent decrease in tissue glycogen content was seen with both enzyme preparations tested. However, the reduction of glycogen following treatment with tgGAA was less than that observed with rhGAA. Interestingly, GAA activity measurements indicated that higher levels of enzyme were present in the tissues of mice treated with tgGAA compared to the levels detected in the tissues of the rhGAA treated mice. This finding suggests that tgGAA may be targeting to cell types other than glycogen containing myocytes within these tissues, or is less efficient at targeting to the lysosomes within the cell. However, Western blot analysis demonstrated that both enzyme preparations tested were fully processed in the tissues homogenates examined, indicating that the different enzyme preparations are reaching the lysosomes. In conclusion, the transgenic and CHO cell derived GAA enzyme both clear glycogen in a dose-dependent manner from the tissues of Pompe mice, although under the conditions of this study, the reduction in glycogen was more significant in mice treated with rhGAA produced in CHO cells.

**A Phase II Open-Label Clinical Study of Efficacy and Safety of Recombinant Human N-Acetylgalactosamine 4-Sulfatase (rhASB) Enzyme Replacement Therapy in Patients With Mucopolysaccharidosis VI (Maroteaux-Lamy Syndrome).** *P. Harmatz*<sup>1</sup>, *D. Ketteridge*<sup>2</sup>, *R. Steiner*<sup>3</sup>, *J. Simon*<sup>1</sup>, *J. Waterson*<sup>1</sup>, *S. Oates*<sup>2</sup>, *R. Giugliani*<sup>4</sup>, *I. Schwartz*<sup>4</sup>, *N. Guffon*<sup>5</sup>, *C. Sa Miranda*<sup>6</sup>, *L. Keppen*<sup>7</sup>, *E. Leao Teles*<sup>8</sup>, *J. Hopwood*<sup>2</sup>. 1) Children's Hosp & Research Ctr Oakland, CA, USA; 2) Women's & Children's Hosp, Adelaide, Australia; 3) Oregon Health & Science U, USA; 4) Hosp de Clinicas de Porto Alegre, Brazil; 5) Hosp Edouard Herriot, France; 6) Inst de Biologia Molecular e Celular, Porto, Portugal; 7) U South Dakota, USA; 8) Hosp de Sao Joao, Porto, Portugal.

Mucopolysaccharidosis VI (MPS VI; Maroteaux-Lamy syndrome) is a lysosomal storage disease caused by a deficiency of the enzyme N-acetylgalactosamine 4-sulfatase (ASB) leading to a progressive disorder with multiple tissue and organ involvement. A Phase II, open-label clinical study to evaluate the efficacy and safety of weekly treatment with 1.0 mg/kg of rhASB for 48 weeks was recently completed. Week 24 data is presented here; week 48 data will also be reported. Ten MPS VI patients were enrolled at two sites (age 6-22 yrs; 3 males). The average improvement in the 6 minute timepoint of the 12 Minute Walk Test was 62% and the 12 minute timepoint was 98%. The average improvement in the 3 minute stair climb was 110%. Joint Pain and Stiffness Questionnaire scores improved 57% and 54%, respectively. Small improvements in passive and active shoulder range of motion were noted with greatest improvements seen in patients with the most restriction at baseline. Biochemical response was documented by reduction in urinary excretion of glycosaminoglycans (GAG) by 71%. ERT was well tolerated. 13 AEs were reported: 5 during infusion and an additional 8 on the day of the infusion. In addition, 7 SAEs, 6 unrelated to drug and 1 possibly related to drug, were noted. 8 subjects developed antibodies to rhASB that did not significantly affect urinary GAG levels. In conclusion, rhASB treatment was well-tolerated, urinary GAG decreased in all patients, and positive clinical responses were present with the greatest improvements observed in assessments of endurance. (Sponsored by BioMarin Pharmaceutical Inc., Novato, CA).

**Short term enzyme replacement therapy in the mouse model of the lysosomal disease sialidosis.** *D. Wang, E. Bonten, L. Mann, T. Walker, A. d'Azzo.* Genetics and Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN.

Sialidosis is a lysosomal storage disorder (LSD) characterized by deficient lysosomal neuraminidase (NEU1) activity and aberrant accumulation of sialylated glycoconjugates. Cells of the reticuloendothelial system are primarily affected. *Neu1* knockout mice develop clinical abnormalities reminiscent of early-onset sialidosis in children, including severe nephropathy, progressive edema, splenomegaly, kyphosis. These mice provide a useful model for testing the efficacy of novel therapeutic strategies for this heritable disorder.

Enzyme replacement therapy (ERT) has proved effective for treating the systemic manifestations associated with LSDs. We have tested the efficacy of this therapeutic approach in the *Neu1* knockout mice by using recombinant enzymes produced in insect cells. Glycoproteins expressed in these cells carry carbohydrates modified to expose mainly core type oligosaccharides and, therefore, are poised to be internalized by macrophages via mannose receptors.

Given the dependence of NEU1 on PPCA for activation and stability, we speculate that injection with both enzymes may afford a better correction of the phenotype. We have therefore overexpressed murine Neu1 and human PPCA in insect cells using a baculovirus expression system and subsequently purified them. One-month-old *Neu1*<sup>-/-</sup> mice were injected intravenously with both recombinant enzymes twice a week for a period of two weeks. Injected enzymes were effectively taken up by many tissues. The half lives of the injected enzymes ranged from 1-4d, depending on the tissue. Restoration of enzyme activity in different tissues was achieved after treatment. Lysosomal storage was reduced in many tissues with significant correction observed in spleen, liver and kidney. Therefore, ERT with baculovirus-expressed enzyme may be an effective treatment for patients with sialidosis and possibly for other LSDs primarily involving the reticuloendothelial system. (Supported in part by NIH grants DK52025, GM60905 and Cancer Core Grant CA21765)

**Replacing a deficient enzyme in Pompe disease: two approaches, one result.** *N. Raben*<sup>1</sup>, *M. Danon*<sup>2</sup>, *A. Gilbert*<sup>1</sup>, *K. Nagaraju*<sup>3</sup>, *P. Plotz*<sup>1</sup>. 1) NIAMS, ARB, NIH, Bethesda, MD; 2) Hennepin Medical Center, University of Minnesota, Minneapolis, MN; 3) Division of Rheumatology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

The success of enzyme replacement therapy (ERT) in type I Gaucher disease, a deficiency of lysosomal glucocerebrosidase, has stimulated efforts to develop similar approaches for other lysosomal disorders. Repeating this success, however, has proved difficult in Pompe disease [glycogen storage disease type II; deficiency of glycogen-degrading lysosomal acid alpha-glucosidase (GAA)] - the only lysosomal storage disease in which muscle is the principal target tissue for ERT. We have evaluated the efficacy of recombinant human GAA (rhGAA from Genzyme Corporation, Framingham, MA) in a tolerant GAA knockout mouse strain. Near complete reversal of pathology was achieved in heart, while only a modest effect was observed in skeletal muscle, even in mice treated with a high dose of the enzyme. After 5 months of therapy, glycogen reduction in skeletal muscle was less than 50%, and the undegraded glycogen remained in proliferating lysosomes as shown by increased immunoreactivity for LAMP-1/LAMP-2 in glycogen-containing lysosomes. These data closely paralleled the results of inducing the GAA transgene in knockout mice - complete clearance of glycogen in the heart but strikingly uneven clearance in skeletal muscle following hepatic secretion of the enzyme - suggesting that the problem lies in the target tissue itself. We have shown that skeletal muscle has a lower density of the essential transporter, the cation-independent mannose 6-phosphate receptor (CI-MPR), than cardiac muscle. Furthermore, histochemical analysis showed increased CI-MPR immunoreactivity in slow-twitch oxidative type 1 muscle fibers, which cleared glycogen more efficiently compared to fast-twitch fibers despite their higher level of glycogen accumulation. These studies suggest that treating myopathy with ERT may require high doses of exogenous enzyme or an entirely different approach.

**The Clinical Benefit of Aldurazyme (laronidase) for the Treatment of MPS I.** *G.M. Pastores<sup>1</sup>, J.E. Wraith<sup>2</sup>, L.A. Clarke<sup>3</sup>, M. Beck<sup>4</sup>, E.H. Kolodny<sup>1</sup>, J. Muenzer<sup>5</sup>, G.F. Cox<sup>6</sup>, A.M. Skrinar<sup>6</sup>.* 1) NYU, NY, NY; 2) U Manchester, Manchester, UK; 3) UBC, Vancouver, Canada; 4) U Mainz, Mainz, GER; 5) UNC, Chapel Hill, NC; 6) Genzyme Corporation, Cambridge, MA.

**Objective:** Mucopolysaccharidosis (MPS I) is an autosomal recessive disorder caused by a deficiency of a lysosomal enzyme, -L-iduronidase, that results in the progressive accumulation of glycosaminoglycans. The spectrum of resulting MPS-related symptoms leads to functional impairment in activities of daily living, particularly in the mobility and self-care domains. The objective of this study was to document improvements in the physical function of MPS I patients treated with Aldurazyme (recombinant human alpha-L-iduronidase). **Methods:** 45 MPS I patients (ages 6-43) were randomized to participate in a Phase 3, double-blind, placebo-controlled study of Aldurazyme for 26 weeks, followed by an ongoing, open-label extension study. Improvements in physical function were assessed using the Disability Index (DI) of the Health Assessment Questionnaire (HAQ) and the Child Health Assessment Questionnaire (CHAQ). The DI scale ranges from 0 to 3, with a score of 3 representing the maximum level of disability. A reduction in score is associated with improved physical function. A change of -0.22 in HAQ DI scores has been established as the minimum clinically important difference (MCID). When mean changes in a treatment group exceed the MCID, it is expected that the majority of patients in this group will achieve clinically meaningful improvements. **Results:** Mean reductions of -0.3 were observed for the Aldurazyme/Aldurazyme group after 26 weeks of treatment in the double-blind study. Mean reductions of -0.2 were observed for the Placebo/Aldurazyme group after 24 weeks of treatment in the open-label extension study. These improvements in physical function have been maintained for an additional 24 weeks of treatment in the open-label extension study. **Conclusion:** MPS I patients treated with Aldurazyme showed mean reductions in CHAQ/HAQ DI scores consistent with MCID values. This suggests improvements in daily functioning that are both perceptible and considered clinically meaningful to the patient.

**Long Term Enzyme Replacement Therapy for Fabry Disease.** *W.R. Wilcox<sup>1</sup>, M. Banikazemi<sup>2</sup>, N. Guffon<sup>3</sup>, P. Lee<sup>4</sup>, G.E. Linthorst<sup>5</sup>, S. Waldek<sup>6</sup>, D.P. Germain<sup>7</sup>, R.J. Desnick<sup>2</sup>, for the International Collaborative Group on Fabry Disease.* 1) Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) Mt Sinai School of Medicine, New York, NY; 3) Hôpital Edouard Herriot, Lyon, France; 4) National Hospital for Neurology and Neurosurgery, London, UK; 5) Academic Medical Center, Amsterdam, The Netherlands; 6) Hope Hospital, Manchester, UK; 7) Hôpital Européen Georges Pompidou, Paris, France.

Fabry disease results from deficient activity of  $\alpha$ -galactosidase A ( $\alpha$ -GAL A) and subsequent accumulation of neutral glycosphingolipids, predominantly globotriaosylceramide (GL-3). All 58 patients who were enrolled in a 20 week Phase 3 Double Blind Placebo Controlled Study of recombinant human  $\alpha$ -galactosidase A (Fabrazyme (agalsidase beta) Genzyme Corporation) have subsequently received r-hGAL (1 mg/kg IV biweekly) in an ongoing open-label extension study for an additional 30 months. As of the 30 month assessment, 50 patients remained in the study. Plasma GL-3 levels were evaluated using a new mass spectrometry assay. There was a sustained reduction in plasma GL-3 levels at 30 months of therapy. Mean serum creatinine, estimated GFR and proteinuria remained stable for up to 36 months of treatment, suggesting stabilization of renal disease. Thirty-nine of 40 (98%) patients who underwent skin biopsies at 30 months had a skin capillary endothelial cell score of zero, demonstrating sustained clearance of GL-3 from the endothelial cells. IgG antibody formation, evaluated using ELISA and a confirmatory radioimmunoprecipitation (RIP) assay, occurred in 52/57 (89%) patients. As of 30 months, 7 of these seroconverted patients tolerized (no detectable IgG antibody) and 58% of patients demonstrated a four-fold or greater reduction in antibody titer. Seroconversion did not impact reduction of plasma GL-3. The mean infusion time at 30 months was 2.2 hours; only 5 patients had an infusion time >3 hours. In summary, enzyme replacement therapy with r-hGAL continues to demonstrate a favorable safety and efficacy profile.

**Pharmacokinetics of Aldurazyme (laronidase) for the Treatment of MPS I.** *S. Swiedler<sup>1</sup>, J.E. Wraith<sup>2</sup>, L.A. Clarke<sup>3</sup>, W. Kramer<sup>4</sup>, K. Walton-Bowen<sup>5</sup>, G.F. Cox<sup>5</sup>.* 1) BioMarin Pharmaceuticals, Novato, CA; 2) University of Manchester, Manchester, UK; 3) University of British Columbia, Vancouver, CA; 4) Kramer Consulting LLC, North Potomac, MD; 5) Genzyme Corporation, Cambridge, MA.

**Objective:** To evaluate the pharmacokinetics (PK) profile of laronidase and the impact of antibody formation.

**Methods:** PK analysis was performed on a subset of 12 patients with MPS I from the Phase 3 study who received Aldurazyme at 100 U/kg (0.58mg/kg) IV weekly for 26 weeks. Infusions were administered over 4 hours using a ramp-up infusion rate protocol. Plasma laronidase concentrations were measured over 10 hours. PK parameters were estimated for the 1, 12, and 26 infusions using non-compartmental analysis. Values from week infusions 1 and 26 were compared using a paired t-test.

**Results:** The maximal plasma concentration of laronidase ( $C_{max}$ ) increased over time ( $p < 0.001$ ), possibly due to a concomitant 50% reduction in the volume of distribution ( $V_z$ ) ( $p = 0.001$ ). The reduced  $V_z$  also may have accounted for the trend towards a decreased elimination half-life ( $t_{1/2}$ ) over time ( $p = 0.010$ ). An inverse relationship observed between  $V_z$  and anti-laronidase antibody levels at infusions 12 and 26 suggested a causal interaction. Importantly, however, plasma clearance (CL) remained relatively stable after 26 infusions ( $p = 0.253$ ). In association with an infusion with Aldurazyme, plasma laronidase concentrations remained at levels above the half-maximal saturation of uptake into cells (0.7 nM; 0.01 U/mL) for a minimum of 3-9 hours. Pharmacodynamic and clinical measures improved despite antibody formation in most patients receiving laronidase.

**Conclusions:** The PK parameters of laronidase have been determined. Despite an increased  $C_{max}$  and decreased  $V_z$  on the first and 26th treatment, the unchanged CL indicates that the overall irreversible transport of laronidase out of the plasma is not affected by antibodies to laronidase. (Supported by BioMarin/Genzyme LLC).

**Intravenous retroviral gene therapy in seven-week-old dogs with mucopolysaccharidosis (MPS) VII.** *M. Haskins<sup>1</sup>, T. O'Malley<sup>1</sup>, NM. Ellinwood<sup>1</sup>, P. O'Donnell<sup>1</sup>, K. Cullen<sup>1</sup>, L. Xu<sup>2</sup>, R. Mango<sup>2</sup>, KP. Ponder<sup>2</sup>.* 1) University of Pennsylvania, Philadelphia, PA; 2) Washington University, St. Louis, MO.

MPS VII is a lysosomal storage disease due to deficient -glucuronidase (GUSB) activity. We previously reported that IV injection of  $3 \times 10^9$  transducing units (U)/kg of a retroviral vector (RV) expressing GUSB into newborn MPS VII dogs resulted in transduction of hepatocytes, which secreted GUSB into blood. One MPS VII animal that was treated with human hepatocyte growth factor (HGF) prior to neonatal IV injection of  $12 \times 10^9$  U/kg of the same RV (HGF/RV-treated) had dramatically higher serum levels (17,442 U/ml; 60 times normal), possibly due to the HGF or the higher dose of RV. Now an additional dog, not pretreated with HGF, given a log higher dose of RV than the previous HGF-treated dog has stable serum GUSB activity of 108 U/ml (36.3% of normal) 5 months after treatment, indicating that HGF was likely responsible for the extremely high serum GUSB activity in the initial dog. In all 4 of the neonatally treated dogs followed more than 2.5 years, clinical signs were minimal, and provirus and expression of GUSB were detected in peripheral blood indicating transduction of hematopoietic stem cells. Because most MPS patients are not diagnosed until 1-2 years of age, we tested if post-weaning, 7-week-old dogs could be treated by injecting  $1.7-2.2 \times 10^9$  U/kg of RV IV as 4 doses over 4 days. Four dogs were pre-treated with 2.5 mg/kg of HGF (HGF/RV); 3 were only given RV. The HGF/RV- and RV-treated dogs had  $184 \pm 109$  (54% of normal) and  $49.7 \pm 11$  (14% of normal) U/ml of serum GUSB, respectively. None of these dogs has PCR-detectable provirus in peripheral blood. We conclude that 1) 7-week-old dogs can achieve serum GUSB activity that should be associated with an improvement in clinical features, 2) HGF increased transduction over that obtained without HGF, 3) expression was surprisingly high without HGF, and 4) hematopoietic stem cell transduction in the neonatal dogs probably occurred in the liver.

**Long-term correction of ornithine transcarbamylase deficiency by tissue-restricted over-expression using a helper-dependent adenovirus.** *A. Mian*<sup>1</sup>, *V. Mane*<sup>1</sup>, *P. Ng*<sup>1</sup>, *M. Finegold*<sup>2</sup>, *W.O. Brien*<sup>1</sup>, *A. Beaudet*<sup>1</sup>, *B. Lee*<sup>1, 3</sup>. 1) Molecular and Human Genetics; 2) Pathology; 3) Howard Hughes Medical Institute. Baylor College of Medicine, Houston, TX.

The urea cycle disorders (UCDs) are important models for developing gene replacement therapy for liver diseases. Long-term correction of the most common UCD, ornithine transcarbamylase (OTC) deficiency, has yet to be achieved in clinical or preclinical settings. The single human clinical trial for OTC deficiency using an early generation adenovirus (Ad) failed to show any biochemical correction. In OTC-deficient mice, an E1/E2-deleted Ad vector expressing the mouse OTC gene, but not the human, was therapeutic for 2 months. By combining tissue-restricted expression of human OTC, a post-transcriptional enhancer and the less immunogenic helper-dependent adenoviral vector, we achieved metabolic correction of OTC-deficient mice for greater than 6 months. This was demonstrated by normalized orotic aciduria, normal hepatic enzyme activity and elevated OTC protein levels, but without chronic toxicity. Over-expressing the human protein may have overcome a proposed kinetic block at the level of mitochondrial import. Taken together, these data represent an important approach for treating cell autonomous human inborn errors of hepatocyte metabolism that require high level transduction and gene expression for clinical correction.

**Targeting In Vivo Gene Correction Using Designed DNA-Binding Proteins.** *M.C. Holmes, F.D. Urnov, J.C. Miller, A.C. Jamieson, Y.-L. Lee, S. Augustus, T. Martin, C.O. Pabo.* Sangamo BioSciences, Inc., Richmond, CA.

Progress in understanding the molecular basis of human monogenic disorders has not been followed by an increase in efficient strategies for therapeutic intervention. Many human genetic disorders are caused by hypomorphic or null mutations in genes, and restoration of even a single wild-type allele to the cell can be curative. In practice, however, the human genome is resistant to gene correction or replacement by homologous recombination (HR). As a consequence, therapeutic transgenes carrying wild-type sequences integrate at nonnative loci, and this misintegration results in aberrant genome behavior or in the failure of the transgene to express properly. We describe our recent studies towards developing a novel and efficient method for obtaining in vivo gene correction at genetic loci involved in monogenic disorders. Using engineered Zinc Finger Proteins (ZFPs) fused to the catalytic domain of the type IIS restriction endonuclease, FokI, we can introduce a double strand break (DSB) at any desired region of the human genome. Engineered ZFPs can be designed to bind virtually any DNA sequence with high affinity and specificity, enabling the tethered catalytic domain of FokI to cleave the DNA at precise sites in the genome. The introduction of targeted DSBs stimulate a potent repair pathway that facilitates the replacement of the DNA sequence at the break point with genetic information from a homologous DNA donor molecule, and thereby, provide a mechanism by which genetic errors can be permanently corrected via HR-mediated Gene Correction. In model systems, ZFP-FokI chimeras have been shown to massively potentiate HR within integrated reporter genes containing inserted ZFP-binding sites. Therefore, the use of engineered ZFP-FokI proteins to specifically target and cleave a unique region of the genome offers an unprecedented opportunity to enable locus-specific, high-efficiency gene correction for the repairing of genetic lesions encoding monogenic disorders. We will describe our recent studies toward developing a novel and efficient method for in vivo gene correction.

**Recombinant adenovirus mediates human glycerol kinase expression in glycerol kinase knock-out mice. N.**

*Kuwada<sup>1</sup>, K. Nagano<sup>1,2</sup>, M. Kumar<sup>1</sup>, K.M. Dipple<sup>1</sup>, E.R.B. McCabe<sup>1,3</sup>.* 1) Dept of Pediatrics, David Geffen Sch Med at UCLA and Mattel Children's Hosp at UCLA, LA, CA; 2) Dept of Pediatrics Kumamoto Univ Sch Med, Kumamoto, Japan; 3) Dept of Human Genetics, David Geffen Sch Med at UCLA, LA, CA.

Isolated X-linked glycerol kinase deficiency (GKD) may be either symptomatic or asymptomatic, and we have previously shown for point mutations that the GK genotype does not predict phenotype. A GK knock-out (KO) mouse model (Huq, et.al. Hum. Molec. Genet. 6:1997) will permit improved understanding of pathogenesis and the influence of modifier genes on the phenotype. The early death of affected murine males has limited the characterization of GKD pathogenesis. The purpose of this work was to rescue the affected males in order to investigate more thoroughly their phenotypes. We constructed an E1/E3 deleted adenovirus vector (Adeno-X: Clontech, Palo Alto, CA) with the human GK gene under control of cytomegalovirus promoter (Adeno-XGK). We injected Adeno-XGK into the superficial temporal vein of wild type (WT) and KO pups within 24 hours of birth. At ~45-48 hours post injection, the injected pups were sacrificed and the liver, kidney, heart and brown fat were isolated. The GK activity in the livers of KO mice injected with  $2.1 \times 10^8$  pfu of Adeno-XGK was 4.10.8% of WT pup's liver (n=4). When injected with  $3.0 \times 10^8$  pfu and  $6.0 \times 10^8$  pfu, the hepatic activity in KO mice was 13.422.0% (n=4) and 35.09.3% (n=3) of WT, respectively. GK activity in the other organs injected with  $6.0 \times 10^8$  pfu of Adeno-XGK were: brown fat 0.4% (n=1); kidney 1.0% (n=3); and heart 11.3% (n=1). Plasma from pups injected with  $2.1 \times 10^8$  pfu of Adeno-XGK was analyzed biochemically: plasma glycerol and free fatty acid concentrations remained high and triglyceride concentrations remained low. KO pups injected with  $6.0 \times 10^8$  pfu Adeno-XGK did not survive significantly longer, nor have significant changes in body weight compared with non-treated KO pups. We conclude that correction of hepatic GK activity by 30% is not adequate that rescue the GK KO pups. We speculate that much higher liver GK activity will have to be achieved to rescue the affected mice or expression of GK will be required in other tissue(s), for example in brown fat.

**Generation and analysis of a transgenic mouse model with a modified human rhodopsin replacement gene with therapeutic potential.** *M. O'Reilly, B. O'Neill, A. Palfi, P. Kenna, P. Humphries, J. Farrar.* Department of Genetics, Trinity College, Dublin, Ireland.

A transgenic mouse was generated that carries a human rhodopsin gene under the control of the mouse rhodopsin promoter. This rhodopsin gene encodes an identical amino acid sequence to the wild type human rhodopsin gene but has a number of nucleotide differences. These differences are designed, using the degeneracy of the genetic code, to allow transcripts from this modified rhodopsin gene when transcribed to escape cleavage by a ribozyme or siRNA molecules that target that particular site. The transgenic mice were bred onto a mouse rhodopsin knockout ( $\rho\text{-/-}$ ) background. These mice were analysed using electroretinography and histology, comparing function and retinal thickness to that of wild type mice. The modified human rhodopsin gene rescues disease pathology in a  $\rho\text{-/-}$  mouse. This is a useful animal model to study the efficacy of the gene replacement element of a therapeutic strategy for autosomal dominant retinitis pigmentosa (adRP). This model supports the idea of a mutation independent suppression and replacement therapeutic strategy for adRP, and the general strategy is also applicable to other dominantly inherited diseases. Suppression agents such as ribozymes and siRNA molecules that cleave wild type human rhodopsin transcripts at the target site should not cleave the replacement gene transcript. The suppression element of the strategy involves downregulation of gene expression in a mutation independent manner ensuring its application to patients with any mutation in a given gene. The work to be presented here demonstrates that the replacement element of the strategy works well in an animal model.

**Human CBS (Cystathionine -synthase) gene transfer mediated by recombinant adeno-associated virus vector.**

*E.S. Park*<sup>1</sup>, *S.C. Jung*<sup>2</sup>, *H.J. Oh*<sup>2</sup>, *C.H. Lee*<sup>1</sup>, *H.W. Lee*<sup>1</sup>, *J.S. Lee*<sup>3</sup>. 1) BK21 project for Medical Science, Yonsei University, Seoul, Korea; 2) Division of Genetic Disease, National Institute of Health, Seoul, Korea; 3) Department of Clinical Genetics, Yonsei University College of Medicine, Seoul, Korea.

Cystathionine -synthase (CBS) deficiency in human is the most common cause of Homocystinuria, a rare autosomal recessive disease. Patients with this disorder have premature arteriosclerosis and thrombosis, mental retardation, ectopia lentis, osteoporosis, skeletal abnormalities, which are resulted by elevated plasma homocysteine levels. About 50% of patients with CBS deficiency respond to pyridoxine therapy with a marked reduction in plasma total homocysteine, whereas the remaining subjects respond poorly. At least 60 different mutations of the gene have been described, with I278T and G307S as the most common. Interestingly, a number of patient-derived mutations in the CBS catalytic region can be functionally suppressed by either deletion of the c-terminal regulatory region or specific point mutation in this region. For the gene therapy of Homocystinuria, we constructed human CBS cDNA containing recombinant AAV vector (rAAV-hCBS). We used three kinds of hCBS cDNA, one was normal CBS cDNA, two were mutated hCBS cDNA which were deleted or mutated in the region of catalytic domain. We used adenovirus-free production system for the production of rAAV-hCBS vectors. Three kinds of rAAV-hCBS vectors were transduced into NIH3T3 for in vitro evaluation. Western blot analysis using anti-hCBS antibody demonstrated that all rAAV-hCBS vectors expressed high levels of Cystathionine -synthase enzyme. CBS enzyme activities were checked using TLC. There were no much differences among those three vectors. Following the in vitro experiment, we evaluated the transgene expression in homocystinuria mice. We administered an recombinant adeno-associated virus vector encoding hCBS to homocystinuria mice on day 6 of life via IP. The life span of mice was prolonged about 2 times than untreated disease mice. Studies are currently underway to further evaluate our vector in homocystinuria mice.

**Chimeric RNA and ethylene bridged nucleic acid-induced skipping of the exon retaining a stop mutation and led to dystrophin expression in dystrophin-deficient myocytes of Duchenne muscular dystrophy.** *Y. Takeshima<sup>1</sup>, A. Surono<sup>1</sup>, H. Wada<sup>2</sup>, M. Yagi<sup>1</sup>, M. Takagi<sup>3</sup>, M. Koizumi<sup>3</sup>, M. Matsuo<sup>1</sup>.* 1) Dept Pediatrics, Kobe Univ Grad Sch Med, Kobe, Hyogo, Japan; 2) Dept Pediatrics, Sakura Ryoikuen Hospital, Sanda, Hyogo, Japan; 3) Exploratory Chemistry Research Laboratories, Sankyo Co., Ltd., Tokyo, Japan.

Duchenne muscular dystrophy (DMD) is a fatal muscle wasting disease and characterized by dystrophin deficiency in skeletal muscle. Many studies have been done to express dystrophin in myocytes of DMD and modulation of dystrophin mRNA has been proposed to induce dystrophin expression. Here, we report that dystrophin was expressed in myocytes of DMD when skipping of the exon retaining a nonsense mutation was induced by transfection of antisense oligonucleotide. We identified a single nucleotide change in exon 41 of the dystrophin gene from a Japanese DMD case (C5899T) and the mutation converted a codon of arginine to a stop codon (R1967X), resulting dystrophin deficiency. Remarkably, two mRNAs with normal exon content or skipping of exon 41 was disclosed in lymphocytes while not in skeletal muscle. Since the novel mRNA lacking exon 41 was expected to produce dystrophin, artificial induction of exon 41 skipping was examined by using antisense oligonucleotide. As a part of antisense oligonucleotide sequence, we employed a novel modified nucleoside, named 2-0,4-C-ethylene-bridge nucleic acid (ENA) that have a high binding affinity for the complementary RNA strand and are more nuclease-resistant. An 18-mer chimeric RNA and ethylene bridged nucleic acid was sufficient to induce exon 41 skipping completely in the patients cultured myocytes. Furthermore, dystrophin expression was observed in over 80% of cells. The protein was detectable at significant level up to a week after transfection.

Our results demonstrated that skipping of exon 41 could be induced with an antisense oligonucleotide with ENA modification and this is a vital progress towards a clinically applicable for the treatment of diseases by using antisense ENA.

**Expression of the coxsackie-adenovirus receptor (CAR) in the non-neoplastic genito-urinary tract and its implications for gene therapy using adenoviral vectors.** *J. Simko*<sup>1,2</sup>, *V. Weinberg*<sup>2</sup>, *P. Carroll*<sup>2,3</sup>, *F. McCormick*<sup>2,4</sup>, *K.A. Rauen*<sup>2,5</sup>. 1) Pathology, UC San Francisco, CA; 2) Comprehensive Cancer Center, UC San Francisco, CA; 3) Urology, UC San Francisco, CA; 4) Microbiology and Immunology, UC San Francisco, CA; 5) Pediatrics, UC San Francisco, CA.

Adenoviral-based gene therapies are dependent on infectivity of the target tissue through the coxsackie-adenovirus receptor, CAR, which is the primary adenoviral receptor. Its presence is a determining factor in efficient adenoviral infection. CAR serves as the site for attachment of human adenovirus via the fiber protein to the target cell membrane. CAR is found in the tight junctions between epithelia cells and its physiologic function is believed to be important in cell-cell interaction and adhesion. We have recently demonstrated that metastatic prostate carcinoma expresses high levels of CAR, especially relative to primary tumors confined to the prostate. While studies of CAR membrane expression levels in other tumors of the genito-urinary (GU) tract are ongoing, we have evaluated the benign epithelia of the male GU system to obtain a baseline of CAR expression in these tissues. The rationale for the study is several fold: to examine the normal physiologic expression of this cell adhesion molecule so as to provide a baseline for comparing relative CAR expression levels between normal and tumor tissue, to understand how much virus may be absorbed by the benign tissue component during therapy and to know whether toxicity of normal tissues may be a serious consideration due to possible infectivity. We evaluated CAR expression in normal formalin-fixed, paraffin-embedded tissues of the male GU system which included prostate, kidney, ureter, urinary bladder, seminal vesicle, urethra and testis. The intensity of CAR staining for the normal specimens was grouped into four categories. All prostate, bladder, kidney, ureter and testis tissues demonstrated strong staining. Reduced staining was present in seminal vesicle and urethral specimens. These results indicate that the intrinsic expression level of CAR throughout the epithelia of the GU tract is high, suggesting that these tissues are potential targets for infectivity.

**AAV vector mediated gene therapy of Fabry knockout mice.** *T. Shimada*<sup>1</sup>, *K. Zenri*<sup>1</sup>, *K. Ogawa*<sup>1</sup>, *H. Takahashi*<sup>1</sup>, *Y. Hirai*<sup>1</sup>, *Y. Seino*<sup>2</sup>, *Y. Fukuda*<sup>3</sup>. 1) Dept Biochem, Molec Biol,; 2) Dept Internal Med,; 3) Dept Pathology, Nippon Medical Sch, Tokyo, Japan.

Fabry disease is an X-linked inherited metabolic disorder due to deficiency of a lysosomal enzyme  $\alpha$ -galactosidase A ( $\alpha$ -gal A). Undigested glycolipids such as globotriaosylceramide (Gb3) accumulate in systemic organs, resulting in renal and cardiac failure with increasing age.  $\alpha$ -gal A knock-out mice are considered useful for studying pathogenesis and therapeutic strategies of Fabry disease. We have recently demonstrated that AAV mediated expression of  $\alpha$ -gal A in muscle increases the enzyme level in serum and efficiently cleared Gb3 in all organs of Fabry mice. In the present study, we further evaluated histological and functional changes of Fabry mice. The therapeutic AAV vector was injected into the right quadriceps muscles of 12-week-old Fabry mice. The animals were sacrificed 25-32 weeks after vector injection. HE staining of the kidney of untreated Fabry mice showed marked swelling of glomeruli and thickening of the basement membrane. Electron microscopic examination showed that lipid inclusions with lamellar structures were accumulated in the swollen glomerular epithelial cells and tubular epithelial cells in untreated mice. Despite of such significant histological changes, the values of BUN and creatinine were within the normal range. AAV vector treatment normalized the mean diameter of glomeruli and significantly decreased the number of lipid granules. Echocardiographic examination revealed that the gene therapy approach is effective in the improvement of structural and functional parameters, including intraventricular septal thickness (IVST), posterior wall thickness (PWT), left ventricular ejection fraction (LVEF), early to late diastolic transmitral velocity ratio (E/A). These results indicate that accumulated Gb3 induces various histological changes in kidney and heart. But these defects are still reversible at this age and can be treated by AAV vector injection. AAV mediated gene therapy at an early stage may be useful for prevention of end-stage renal and cardiac failure.

**Antisense therapy for DMD in patient cells and mice.** *G.J.B. van Ommen, A. Aartsma-Rus, M. Bremmer-Bout, W.E. Kaman, E.J. de Meijer, A.A.M. Janson, J.T. den Dunnen, J.C.T. van Deutekom.* Department of Human Genetics, Leiden University Medical Center, The Netherlands.

Severe progressive Duchenne muscular dystrophy (DMD) is caused by frame-shifting mutations in the DMD gene. We are targeting adjacent exons to convert DMD into the nearest larger BMD mutation. Using antisense oligoribonucleotides (AONs) directed to exon-internal sequences, we are now able to induce 80-90% skipping of 19 different exons which would correct the DMD mutation in over 75% of patients registered in the Leiden DMD-database ([www.dmd.nl](http://www.dmd.nl)). We have presently validated the therapeutic potential and wide applicability of this strategy in cultured myofibers of 11 DMD patients affected by different deletion and nonsense mutations. Also combined skipping of two exons is efficiently achieved. Dystrophin is detectable as soon as 16 hours post-transfection, increased to significant levels at the membrane within 2 days, and was maintained for at least a week. Proper protein function was further suggested by the restored membranal expression of 4 associated proteins from the dystrophin-glycoprotein complex. Extensive *in vivo* studies in animal models, aimed amongst others at administration and safety, will be necessary prior to clinical trials with DMD patients. We have embarked on these studies and now also achieved *in vivo* exon 46 skipping in mice. Following intramuscular injections of mouse-specific exon 46 AON, exon 46 skipping occurred in a dose-dependent manner. The skipping increased gradually for two weeks and was still detectable after four weeks. We are now developing an *in vivo* exon-skip model system based on the human DMD gene, in transgenic hDMD mice carrying an integral copy of a properly expressed, full-sized 2.5 Mb human DMD gene. Thus, human DMD exon 46 has successfully been targeted *in vivo* in the mouse. Different human DMD-causing exon deletions are now being introduced in this hDMD mouse model. These will facilitate studies on evaluating different antisense AON analogs and delivery systems to ultimately establish a safe and most efficient method suitable for future clinical applications.

**The ataxin-2 (SCA2) knockout mouse phenotype: lack of neurodegeneration and development of marked obesity.**

*K.P. Figueroa*<sup>1</sup>, *A. Nechiporuk*<sup>2</sup>, *T.R. Kiehl*<sup>1</sup>, *D. Huynh*<sup>1</sup>, *X. Xu*<sup>1</sup>, *S.M. Pulst*<sup>1</sup>. 1) Burns and Allen Research Institute and Division of Neurology, Cedars-Sinai Medical Center, Departments of Medicine and Neurobiology, UCLA School of Medicine, Los Angeles, CA; 2) Department of Cardiology and Cell Biology, Harvard Medical School, Boston, MA.

Spinocerebellar ataxia type 2 (SCA2) is a human neurodegenerative disorder caused by expansion of a polyglutamine repeat in the ataxin-2 gene. To elucidate the normal function of ataxin-2 and to determine whether loss-of-function contributes to SCA2 neurodegeneration, we generated a mouse model with a targeted deletion in the *SCA2* gene by homologous recombination in a mixed C57B6/129J background. Deficiency of ataxin-2 was confirmed by Northern and Western blot analyses. Despite embryonic lethality of ataxin-2 deficiency in *C. elegans* and the widespread expression of ataxin-2 during rodent development, *SCA2* <sup>-/-</sup> mice were viable and had no obvious defects or increased morbidity. Although *SCA2* <sup>-/-</sup> mice had normal body weight at birth, we observed subsequent excessive weight gain. At 6 months, the average body weight of *SCA2* <sup>-/-</sup> mice was 26% more than that of wildtype, but we observed no differences in *SCA2* <sup>+/-</sup> mice. Controlled feeding experiments demonstrated that obesity was the result of increased food intake. When maintained in isolation on a restricted diet, no differences in food intake or weight were observed for the three genotypes. However, when provided with an *ad libitum* diet, heterozygote and homozygote mice showed increased food intake accompanied by increased weight. Average weekly food intake was 4.45 g for wildtypes, 5.60 g for heterozygotes, and 6.0 g for knockout mice ( $p < 0.01$ ). Average weekly weight gain was 0.59 g for wildtypes, 0.65 g for heterozygotes, and 0.79 for knockout mice under *ad libitum* conditions ( $p < 0.01$ ). These results suggest that obesity observed in *SCA2* <sup>-/-</sup> mice is predominantly the result of hyperphagia and not of primary metabolic abnormalities. Intermediate obesity observed in heterozygous animals strongly demonstrates that weight control is highly sensitive to ataxin-2 dosage. Supported by RO1 NS33123, the National Ataxia Foundation and the Carmen and Louis Warschaw Endowment.

**Defective bone marrow homing and engraftment in sialidosis mice.** *G. Yogalingam, E. Bonten, T. Walker, L. Mann, A. d'Azzo.* Genetics & Tumor Cell Biology, St Jude Research Hospital, Memphis, TN.

Lysosomal N-acetyl--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). In lysosomes PPCA, NEU1, -galactosidase and N-acetylgalactosamine-6-sulfatase form a multienzyme complex. Sialidosis is caused by mutations in the *Neu1* locus. The disease is systemic affecting both visceral organs and the nervous system. Knockout *Neu1*<sup>-/-</sup> mice completely lack NEU1 activity and manifest a phenotype similar to type II sialidosis. These mice also develop a pronounced splenomegaly characterized by elevated numbers of hematopoietic precursors, consistent with splenic extramedullary hematopoiesis (EMH). We have generated transgenic mice that ubiquitously over-express NEU1 and PPCA gene sequences. Sub-lethally irradiated *Neu1*<sup>-/-</sup> mice were transplanted with transgenic bone marrow (BM) and analyzed one, three and five months post-bone marrow transplant (BMT). Only three out of sixteen *Neu1*<sup>-/-</sup> mice demonstrated successful BM engraftment. Of the three *Neu1*<sup>-/-</sup> mice that did exhibit successful engraftment, NEU1-expressing transgenic macrophages were identified in all organs, including the central nervous system. In comparison, all five control *PPCA*<sup>-/-</sup> mice and all eight control wild-type mice demonstrated successful engraftment following BMT with transgenic BM. A 52 kDa sialylated proteinase was detected in BM extracellular fluid from *Neu1*<sup>-/-</sup> mice with failed BM engraftment using a glycoprotein detection system and gelatin-zymography which was absent in wild-type mice. We are investigating a possible link between this sialylated proteinase and inactivation of the CXCR4/CXCL12 chemotaxis axis which is critical for the homing and retention of hematopoietic stem cells within the BM niche. These results are suggestive of a serious defect in BM homing and engraftment in *Neu1*<sup>-/-</sup> mice and have important implications for BM mediated therapies in sialidosis patients. (Supported in part by NIH grants DK52025 & GM60905).

**Alpha Mannosidosis: Effective Stabilization of Progressive Neuropsychological Decline by Hematopoietic Stem Cell Transplantation.** *S. Grewal, C. Peters, L. Charnas, L. Lockman, P. Orchard, K. Delaney, S. Abel, W. Krivit, E. Shapiro.* Pediatric Neurology, Neuropsychology and Blood & Marrow Transplantation, University of Minnesota, Minneapolis MN USA.

Alpha-mannosidosis results from an inherited deficiency of lysosomal enzyme alpha-mannosidase. Clinical features include progressive slowing in cognitive development with eventual dementia, and a shortened life span. We report the efficacy of hematopoietic stem cell transplantation (HCT) on stabilization of neuropsychological status. Four patients with alpha-mannosidosis underwent allogeneic HCT at the University of Minnesota. Age at HCT was 6.5, 3.3, 23.1 and 4.1 years respectively. Follow-up after HCT has been from 0.7 to 6 years. All patients show donor engraftment and normalization of leukocyte enzyme activity after HCT. All 4 patients showed considerable slowing of their neurocognitive development prior to HCT. Neuropsychological test results within one month prior to HCT and at last followup are summarized in Table and indicate stabilization (within a standard deviation) or improvement (>1 standard deviation) of function after HCT. No instance of significant loss of function (<1 standard deviation) was observed.

**All scores are standard scores: mean of 100 and standard deviation of 15.**

	Pt 1		Pt 2		Pt 3		Pt 4	
	at HCT	4-5 yr	at HCT	5 yr	at HCT	3 yr	at HCT	0.7 yr
<b>IQ</b>	76	79	85	90	78	72	75	ND
<b>Memory</b>	83	88	93	112	66	79	76	68
<b>Vineland</b>	105	90	77	102	50	80	66	60

**CONCLUSION:** HCT can halt the progressive slowing in development and relative decline in cognitive skills that occurs in alpha-mannosidosis. Early diagnosis and treatment is critical.

**A Biochemical Comparison of Recombinant Human Acid Alpha-Glucosidase Expressed in CHO Cells and Transgenic Rabbits.** *K.L. Lee, J. Baker-Malcom, K. Albee, T. Edmunds, R. Mattaliano.* Therapeutic Protein Research and Development, Genzyme, Framingham, MA.

Pompe disease, a deficiency of the hydrolase acid alpha-glucosidase (GAA), results in the abnormal accumulation of lysosomal glycogen. The severity of Pompe disease generally varies with the age of first clinical presentation and ranges from cardiomyopathy and severe hypotonia (e.g., infantile onset) to severe respiratory failure and proximal muscle weakness (e.g., juveniles, adults). Early phase clinical trials have been conducted or are underway in several patient populations using recombinant human acid alpha-glucosidase purified from the milk of transgenic rabbits (tg-GAA) and from genetically modified CHO cells (rhGAA). Large scale purification of rhGAA using a new CHO cell line has also led to the initiation of two new clinical trials for the treatment of Pompe disease. A biochemical comparison of the CHO-derived rhGAA and the tg-GAA was performed. The predominant GAA species in each preparation was the 110 kDa (precursor) form. N-terminal sequence analysis and LC/MS revealed that the N-terminus of rhGAA was chemically blocked with pyroglutamate, while the N-terminus of tg-GAA was not. The enzyme kinetic parameters for each of the GAA preparations were similar. IEF analysis indicated that both rhGAA and tg-GAA were a heterogeneous mixture of charge isoforms which focused over a broad pI range. The glycans of each of the GAA preparations tested in this study contained different levels of mannose-6-phosphate; however further analysis indicated that the mannose-6-phosphate residues on tg-GAA were modified by N-acetylglucosamine. Surface plasmon resonance studies indicated that rhGAA possessed a higher relative binding affinity for the soluble cation independent mannose-6-phosphate receptor than tg-GAA. Cellular uptake by Pompe fibroblasts was also higher for rhGAA and lower for tg-GAA. Biochemical and functional analyses using surface plasmon resonance and cellular uptake assays suggest that there is a higher quantity of exposed mannose-6-phosphate on rhGAA compared to tg-GAA.

**Increased lipoprotein(a) levels in a subset of patients affected with Fabry disease.** *Y. Herrera-Guzman<sup>1</sup>, M. Cambillau<sup>2</sup>, K. Benistan<sup>1</sup>, S. Sainte-Croix<sup>1</sup>, A. Bissery<sup>3</sup>, D.P. Germain<sup>1</sup>.* 1) Department of Genetics, European Hosp Georges Pompidou, Paris, France; 2) Department of Biochemistry, European Hosp Georges Pompidou, Paris, France; 3) Clinical Research Center, European Hosp Georges Pompidou, Paris, France.

Background: Fabry disease (FD) is an X-linked inborn error of sphingolipid metabolism due to the deficient activity of alpha-galactosidase A, a lysosomal enzyme. It is a multisystemic disorder characterized by progressive renal insufficiency, with added morbidity from cardio- and cerebro-vascular involvement. The recent availability of enzyme replacement therapy (ERT) emphasizes the need for better understanding of the pathogenesis, and for surrogate markers to monitor the efficacy of ERT. Methods: We investigated serum lipoprotein(a) concentrations in 20 consecutive hemizygous males prior to ERT. Results: Elevated serum concentrations of lipoprotein(a) [Lp(a)] (>30 mg/dL) were found in 35% (7/20) of FD patients. These results are to be compared with a prevalence of 4.7 to 10.3% found in numerous studies in the normal population. Increased Lp(a) levels correlated with renal function deterioration and 24h-proteinuria. Discussion: Elevated levels of Lp(a) have been determined to have both atherogenic and thrombogenic effects and various retrospective case-control studies have demonstrated an association of elevated Lp(a) with increased risk for ischemic stroke, coronary heart disease and venous thromboembolism. Although the mechanism and significance of Lp(a) elevation in FD, its relationship with kidney failure and its ability to decrease with ERT deserve further studies, our results suggest that increased Lp(a) level could play a role in the pathogenesis of FD and represent an additional risk factor of thrombosis for a subset of FD patients, especially when combined with hyperhomocysteinemia which has been demonstrated in patients with renal failure or on carbamazepin therapy. Lp(a) should be included in the baseline screening of FD patients. Whether elevated serum levels should prompt to a more aggressive therapy and will normalize on ERT is currently being investigated during follow-up studies.

**Gene therapy of murine G<sub>M1</sub> gangliosidosis by genetically modified bone marrow hematopoietic progenitor cells.**

*R. Sano, A. Tessitore, L. Mann, A. d'Azzo.* Genetics & Tumor Cell Biology, St. Jude Children's Research Hospital Memphis, TN.

-galactosidase (-gal), a lysosomal enzyme involved in the removal of -linked terminal galactosyl residues of many glycoconjugates, is deficient in the neurodegenerative lysosomal disorder G<sub>M1</sub> gangliosidosis (GM1). GM1<sup>-/-</sup> mice closely mimic the most fundamental aspects of the neuropathological and neurochemical abnormalities of the human disorder. Bone marrow progenitor cells have been used as a source of corrective protein because of their ability to repopulate the recipients and to supply functional enzyme to different cells by in trans correction. Hematopoietic progenitors transduced with a murine stem cell virus (MSCV)-based bicistronic retroviral vector overexpressing -gal and the green fluorescent protein (GFP) marker were used for transplantation into sublethally irradiated GM1<sup>-/-</sup> mice. The transduction efficiency of MSCV--gal was 25-89 % prior to the transplantation. -gal expressing bone marrow-derived cells were detected histologically and enzymatically in many tissues including spleen, liver, lung, intestine and kidney after one and three months post transplantation. GFP-expressing cells of the erythroid, myeloid or lymphoid lineage were detected by FACS analysis of peripheral blood samples, collected at different time points after transplantation. The percentage of gated cells expressing GFP varied between 1.20% and 30.98% in erythrocytes, 1.17% and 73.78% in platelets and 16.47% and 69.40% in lymphocytes. In some tissues the restoration of the enzyme activity was close to the wild type mice. Moreover cross-correction experiments using GM1<sup>-/-</sup> human fibroblasts showed the capability of internalization and processing to a mature active form of the enzyme. Taken together these preliminary results have reinforced the feasibility of using ex-vivo gene therapy for the treatment of GM1 (Supported in part by NIH grant DK 52025).

**Treatment approaches for the mouse models of globoid cell leukodystrophy.** *M.A. Rafi<sup>1</sup>, P. Luzzi<sup>1</sup>, M. Zaka<sup>1</sup>, M.A. Passini<sup>3</sup>, M.T. Vanier<sup>2</sup>, J.H. Wolfe<sup>3</sup>, D.A. Wenger<sup>1</sup>.* 1) Dept. Neurology, Thomas Jefferson Univ., Philadelphia, PA; 2) INSERM U189, Lyon-Sud Medical School, Oullins, France; 3) Dept. Pathobiology, School of Veterinary Medicine, Univ. of Pennsylvania, PA.

Globoid cell leukodystrophy (GLD) or Krabbe disease is a neurodegenerative disorder caused by the deficiency of the lysosomal enzyme galactocerebrosidase (GALC). This leads to the accumulation of psychosine, resulting in the death of myelin-producing-cells. The naturally occurring mouse model of this disease, twitcher (twi), and our newly generated transgenic (tg) mice with milder symptoms and a slightly longer life span provide excellent models for evaluating therapeutic strategies. Bone marrow transplantation (BMT) of young twi mice can prolong their lives to about 100 days from 40 days in untreated mice. In an attempt to improve the outcome of BMT, we have subjected our tg mice to BMT following pretreatment of BM cells with IGF-1. We have transplanted more than 80 tg mice. Their lifespan was extended from 50 days up to one year. Although the long-living mice still died with typical neurological symptoms, their psychosine levels were ~40 pmol/mg protein compared to ~ 200 in 50-day-old untreated mice. Pretreatment of BM cells with IGF-1 (50 ng/ml) prior to transplantation reduced the mortality from 80% to about 50% within the first 90 days post-transplantation. In addition, we have cloned mouse GALC cDNA in pZAC2.1, a type-2 adeno-associated viral vector (AAV), packaged in AAV-1, AAV-2 and AAV-5 serotypes. In vitro transduction of mouse neural cells and skin fibroblasts with these vectors showed a 5-6-fold increase in GALC activity. Subsequently, these viral vectors were injected intra-ventricularly or intra-parenchymally into newborn twi mouse brains. Two weeks after injection, frozen sections were stained with anti-GALC antibody, and transduced cells were detected by their bright perinuclear staining. There was some background staining in untransduced areas. Analysis of different brain areas 2 wks after injection revealed a 3 to 4-fold increase in GALC activity in regions near the site of injection. Further improvements are needed to provide more GALC activity to both the CNS and PNS.

**Shifting Mitochondrial DNA Heteroplasmy by Mitochondrially-Targeted Restriction Endonucleases. M.P.**

*Bayona-Bafaluy*<sup>1</sup>, *B.J. Battersby*<sup>2</sup>, *E.A. Shoubridge*<sup>2</sup>, *C.T. Moraes*<sup>1</sup>. 1) Neurology, University of Miami, Miami, FL; 2) Montreal Neurological Institute, Montreal, Quebec, Canada.

Mitochondrial DNA (mtDNA) mutations cause a number of devastating human disorders. Most pathogenic mtDNA mutations are heteroplasmic (i.e. the mutated mtDNA co-exists with the wild-type). The wild-type mtDNA fraction has a strong protective effect, and cellular defects are only observed in the presence of very high percentages of mutated mtDNA. We have developed a system to decrease specific mtDNA haplotypes by expressing a mitochondrially-targeted restriction endonucleases *in vivo*. Such system has the potential to reduce a mutated mtDNA fraction, if a restriction endonuclease site is created by a pathogenic nucleotide alteration. As a model system, we used hepatocytes from heteroplasmic mice harboring mtDNA from both BALB and NZB haplotypes. Among several nucleotide variations, these haplotypes differ by the absence of *Apa*LI sites in the NZB mtDNA and the presence of a single site in the BALB mtDNA. Transfection of mitochondrially-targeted *Apa*LI in these cells resulted in a rapid shift in heteroplasmy towards the NZB mtDNA. To better understand the timing of heteroplasmy shift, we placed the mitochondrially-targeted *Apa*LI gene in a vector system inducible by mifiprestone (RU486). Heteroplasmic hepatocyte clones containing the inducible construct treated with mifeprestone for 72 hours showed a complete shift in heteroplasmy and became homoplasmic for the NZB mtDNA. These results indicate that the heteroplasmy shift triggered by mitochondrially-targeted restriction endonucleases is extremely fast and specific. We are currently analyzing the kinetics of heteroplasmy shift and developing a mouse model to test the system in a live animal.

**MELAS and L-arginine Therapy.** *Y. Koga, Y. Akita, I. Ueki, A. Koga, T. Matsuishi.* Dept Pediatrics & Child Health, Kurume Univ Sch Medicine, Fukuoka, Japan.

**Background:** Based on the hypothesis that the stroke in MELAS are caused by impairment of vasodilation in some segment of the intracerebral artery, we administered L-arginine by infusion or by oral, and evaluate the pharmacological effects. **Methods:** We measured biochemical parameters at interictal, acute phase of 22 MELAS patients and the control subjects. On thirty-four occasions of stroke-like episodes, patients were administered 0.5g/kg/dose L-arginine or a placebo. The clinical symptoms, biochemical parameters (including aminoacids, ADMA, cGMP, and NOx) and intracranial hemodynamics were analyzed before and after administration. After administration of oral L-arginine, we evaluate the pharmacological effects on the frequencies and severity of stroke-like episodes in MELAS. **Results:** The levels of L-arginine (46.6 12.7mol/l), citrulline (23.2 10.2mol/l), and NOx (24.0 9.8mol/l) at acute phase of MELAS are significantly decreased than the control (108.1 27.6, 34.6 8.8, 45.4 30.5 mol/l), however, ADMA (0.45 0.10 mol/l) at acute is not significantly decreased. At 30 minutes after L-arginine infusion, all symptoms suggesting stroke were significantly improved in association with significantly increased levels of L-arginine, citrulline, NOx and cGMP. There was a increased uptake in the infarcted region showing improvement in rCBF in the fresh infarct region. The lactate and L/P ratio were recovered to that of interictal phase at 24 hours after L-arginine infusion. After the oral administration of L-arginine, the frequency and severity of stroke-like episodes are significantly improved. **Conclusions:** L-arginine therapy either intravenous infusion or oral administration improved the clinical symptoms and biochemical parameter, and therefore constitutes a new potential therapy for use in stroke-like episodes in MELAS.

**Transmitochondrial yeast strains containing the complete mouse mitochondrial genome: a new *in vivo* model system for analyzing mammalian nuclear-mitochondrial interactions.** Y. Yoon, M. Koob. Hum. Genet., Univ MN, Minneapolis, MN.

Although a growing number of mutations have been identified throughout the human mitochondrial genome that cause a wide range of devastating diseases and the critical importance of this genome is widely acknowledged, we still know amazingly little about many of the most basic aspects of mammalian mitochondrial molecular genetics. To a large degree detailed analyses of this genome are limited by significant technical hurdles: we still have not learned how to directly modify the mitochondrial genome *in vivo* and modifying the nuclear genes that interact with this genome is difficult. We now report that we have successfully generated strains of *Saccharomyces cerevisiae* that contain the entire mouse mitochondrial genome in their mitochondria rather than their own yeast mtDNA. This is the first report of the stable transfer of an isolated mtDNA genome into the mitochondria of a living organism. Since we can easily engineer the mouse mitochondrial genomes that we introduce into these yeast strains, we have created a new *in vivo* mammalian mitochondrial model system in which we can readily manipulate both the nuclear and mitochondrial sequences encoding mitochondrial molecular processes. Most of these processes are strictly dependent on the interaction between specific mtDNA sequences and the nuclear-encoded mitochondrial gene-products with which they have co-evolved, and so do not occur in otherwise unmodified transmitochondrial yeast. By adding the appropriate mammalian genes for nuclear-encoded mitochondrial proteins to these yeast, we will recapitulate such species-specific mitochondrial processes as transcription and the mammalian form of mtDNA replication in these transmitochondrial yeast strains. We have initiated a project in which we are utilizing this approach to determine the precise contribution from mtDNA sequences and nuclear-encoded proteins to the process of transcription initiation in the mammalian mitochondrial genome. We will present the details of the construction and early characterization of this new transmitochondrial model system as well as the results to date of our analyses of mammalian mitochondrial transcription.

**Natural History of Fabry Disease.** *M. Banikazemi*<sup>1</sup>, *R. Schiffmann*<sup>2</sup>, *J. Bultas*<sup>3</sup>, *G.E. Linthorst*<sup>4</sup>, *S. Packman*<sup>5</sup>, *S.A. Sorensen*<sup>6</sup>, *W.R. Wilcox*<sup>7</sup>, *R.J. Desnick*<sup>1</sup>. 1) Mt Sinai School of Medicine, New York, NY; 2) DMNB, NIH, Bethesda, MD; 3) University Hospital, Prague, Czech Republic; 4) Academic Medical Center, Amsterdam, The Netherlands; 5) Univ of California San Francisco, San Francisco, CA; 6) Univ of Copenhagen, Copenhagen, Denmark; 7) Cedars-Sinai Med Cntr, Los Angeles, CA.

Fabry disease results from deficient activity of  $\alpha$ -galactosidase A. Glycosphingolipids accumulate in cellular lysosomes, resulting in renal failure and cardiac and cerebrovascular complications. The natural history of the disease is not well known. We undertook a multicenter retrospective cohort study of patients with Fabry disease to determine their clinical manifestations. In all, 447 patients enrolled in this study at 27 sites. Mean age at Fabry diagnosis was 24 years for males and 30 years for females, 87% of patients had a family history of Fabry disease and 62% were males. Median plasma  $\alpha$ -galactosidase A levels for males (n=120) and females (n=64) were 0.52 and 3.99 nmol/hr/mL, respectively (normal 2.4-23.1 nmol/hr/mL). Mean duration of follow-up was 12.9 years. Median time between Fabry diagnosis and the first serum creatinine (S. Cr.) measurement was 4.3 years; for 25% of patients, the first S. Cr. value was available 13.9 years or more after diagnosis. The S. Cr. level was  $>1.5$  mg/dL among 25% of patients within 1 year of diagnosis. Once the patient's S. Cr was 1.5 mg/dL, the median time to progression (doubling of S. Cr. or development of end-stage renal disease) was 39 months. Renal function decline was rapid once S. Cr. levels reached 1.5 mg/dL. The mean age for development of cardiovascular events (MI, angina, arrhythmia, CHF, change in cardiac status) was 39 years. Cardiac arrhythmias were the most common cardiovascular events (n=162, 36%). The mean age for development of cerebrovascular events (TIA, stroke) was 42 years. Ischemic stroke was the most common cerebrovascular event (n=40, 9%). Early screening for end-organ damage should be undertaken to prevent complications of Fabry disease. Clinical trials are underway to demonstrate clinical benefit of ERT in patients with Fabry disease.

**PPAR agonist corrects fatty acid oxidation in CPT2-deficient myoblasts.** *J. Bastin, J.P. Bonnefont, A. Munnich, F. Djouadi.* INSERM U393, Hopital Necker, Paris, France.

Carnitine palmitoyltransferase 2 (CPT2) deficiency, one of the most common inborn error of mitochondrial fatty acid -oxidation (FAO) leads to recurrent attacks of rhabdomyolysis triggered by exercise, fasting or infections in childhood, or to serious cardiac damages in the neonatal period. It has been suggested that the severity of the disease is related to the residual enzyme activity and FAO capacity. Thus, we hypothesized that a drug able to stimulate FAO could have beneficial effects on CPT2 deficiency. The peroxisome proliferator-activated receptors (PPARs; and isoforms) are ligand activated nuclear transcriptional factors known to play a critical physiological role as lipid sensors and fatty acid metabolism regulators. Recent data identified PPAR as a potent FAO stimulator in C2C12 myotubes (Cell, 113: 159-170, 2003). This prompted us to test the effects of a PPAR agonist (GW0742, kindly provided by GlaxoSmithKline) on FAO in muscle cells of CPT2-deficient patients. Control or CPT2 deficient myoblasts were isolated from muscle biopsies and oxidation rates of tritiated palmitate (3H-Pal) were measured. CPT2-deficient myoblasts exhibited a 18% decrease in FAO compared to control cells (3.870.3 and 4.750.09 nmol3H-Pal/h/mg prot,  $p < 0.001$ , respectively). An overnight glucose deprivation of myoblasts resulted in an additional 24% decrease in CPT2-deficient cells (2.940.6 nmol3H-Pal/h/mg prot) and did not affect the FAO in control cells. Pretreatment of CPT2-deficient myoblasts with GW0742 (1M), 48h before glucose deprivation completely restored FAO to normal levels. Dose-response experiments (with concentrations ranging from 0.01 to 1 M) demonstrated that this PPAR agonist is very potent, as maximal effect was already reached at 0.01M. These are the first evidence of a possible pharmacological effect on CPT2 activity in myoblasts, and point out PPAR as a key regulator of mitochondrial -oxidation in human muscle cells.

**Controlled trial of Pamidronate in children with types III and IV OI: Lack of effect on motor function and pain.**

*A.D. Letocha<sup>1</sup>, H.L. Cintas<sup>2</sup>, S.M. Paul<sup>2</sup>, N.L. Gerber<sup>2</sup>, J.C. Marini<sup>1</sup>*. 1) BEMB/NICHD/NIH, Bethesda, MD; 2) Dept. of Rehabilitation Medicine, CC/NIH, Bethesda, MD.

Osteogenesis imperfecta, a disorder of type I collagen, causes severe osteoporosis and susceptibility to fractures from minimal trauma. Uncontrolled trials of bisphosphonates reported increased vertebral bone density and height, improved strength and functional level and decreased fractures and bone pain.

We completed a 2-year controlled trial of pamidronate in 18 children ages 6-14 years with types III and IV OI. Children in the treatment group received pamidronate (10 mg/m<sup>2</sup>/day for 3 days every 3 months). All children had quarterly rehabilitation assessments including measurements of function, strength, and pain. All patients were also assigned a home physical therapy regimen.

Treated patients experienced a significant ( $p=0.0003$ ) increase in vertebral BMD z-score compared to controls. They also had significant increases in L2 vertebral height and L1 and L2 area.

Motor skills related to ambulation were assessed with the 10-point BAMF. The treatment group BAMF at initiation was 6.11.8 vs 6.71.9 at 12 months ( $p=0.5$ ). The control group BAMF at the initiation was 6.62 vs 7.021.31 at 12 months ( $p=0.61$ ). Both groups, on average, used maximum gait aids. Manual muscle testing was assessed at each visit as the sum (total points 110) of abdominal, straight leg raise, hip abduction, extension, and flexion, and quadriceps strength. Lower extremity muscle strength did not change. The treatment group score was 67.120.9 at baseline vs 68.619.7 at 12 months ( $p=0.88$ ); control was 74.221.3 at initiation vs 74.614.3 at 12 months ( $p=0.97$ ). There was no significant decrease in pain in treatment or control groups on a 4-point scale.

Some patients reported increased endurance or decreased back pain, but most patients reported no perceptible changes. Thus, in contrast to reports from uncontrolled trials, we found no significant changes in ambulation level, lower extremity strength, or pain in OI children treated with pamidronate.

**Idebenone restores mitochondrial respiratory chain enzyme activities in cardiac muscle in Friedreichs ataxia.** *P. Rustin<sup>1</sup>, D. Bonnet<sup>2</sup>, D. Sidi<sup>2</sup>, A. Munnich<sup>1</sup>, A. Rötig<sup>1</sup>.* 1) INSERM U-393, Hopital Necker Enfants Malades, Paris, France; 2) Service de Cardiologie, Hopital Necker Enfants Malades, Paris, France.

The gene responsible for Friedreichs ataxia (FA) has been shown to encode a small protein, frataxin. Decreased frataxin causes a generalized deficiency of iron-sulphur proteins (ISP). Recent data support the view that frataxin plays a role in the mitochondrial synthesis of ISP that are subsequently delivered to the various compartments of the cell. Decreased amount of ISP can in turn be the target of an oxidative stress resulting from abnormal mitochondrial iron handling. This provided the rationale for an antioxidant treatment by idebenone, a drug already available and devoid of reducing effects on stored iron. A large open trial showed that idebenone had a significant effect on heart hypertrophy and function, but not on the neurological condition. The mechanism by which idebenone protects the heart remained to establish. Repeated endomyocardial biopsy showed the normalization of mitochondrial ISP activities in the first patient who was given idebenone since 1998. After 3 years of idebenone administration, heart hypertrophy had decreased to normal values and heart function had improved. Both the shortening fraction and the left-ventricular outflow obstruction had decreased, and b-adrenergic antagonist administration could be discontinued. After 5 years of idebenone administration, the heart hypertrophy has further decreased and no evidence of heart dysfunction was noted. Investigation of endomyocardial micro-biopsies showed that the activity of ISP was largely restored to normal. The striking recovery of mitochondrial respiratory chain enzyme activities brought about by idebenone parallels the improvement of ultrasound parameters and further supports the efficiency of idebenone in improving cardiomyopathy in FRDA. It also sheds light on the mechanism by which an antioxidant, such as idebenone, can have a spectacular effect on the cardiac dysfunction associated with this genetic disorder.

Program Nr: 2702 from 2003 ASHG Annual Meeting

**Chemically induced modification of DNA dynamics: towards chemogenotherapy in the repeat expansion disorders.** *D.G. Monckton, S. Mustafa, J.P. McAbney, M. Gomes-Pereira.* Institute of Biomedical and Life Sciences, University of Glasgow, Anderson College Building, 56 Dumbarton Road, Glasgow G11 6NU, UK.

Expanded repeats disorders such as myotonic dystrophy and Huntington disease share several features associated with DNA instability. In particular, somatic mosaicism of repeat length is often prominent and tends to be age dependent, tissue specific and expansion biased, properties that likely contribute toward the tissue specificity and progressive nature of the symptoms. However, pathology in these disorders is mediated via a variety of complex and unrelated routes. Thus, therapies aimed at the downstream effects of the expansion will be largely disease-specific. We propose that therapies targeted directly at the repeat mutation may have general utility in these disorders. Specifically, strategies that result in suppression of somatic repeat expansion would be expected to be of therapeutic benefit, whilst reversion of the expanded mutant repeat to within the range observed in the general population would be predicted to be curative. To this end we have determined that expanded repeat mutational dynamics may be modified in whole populations of cells by chronic exposure to genotoxic chemicals in a tissue culture model of unstable DNA. Reagents that result in both acceleration and, more importantly, deceleration of the rate of expansion have been identified. These data establish that drug induced modification of DNA dynamics is possible and suggest that chemogenotherapy presents a possible route to treatment in the repeat expansion disorders.